UNIVERSITAT ROVIRA I VIRGILI

# HEALTH IMPACT OF INSECT CONSUMPTION THROUGH THEIR INTERACTION WITH GASTROINTESTINAL TRACT: EXPLORING THE ROLE OF INTESTINAL TASTE RECEPTORS

**Helena Segú Matamoros**

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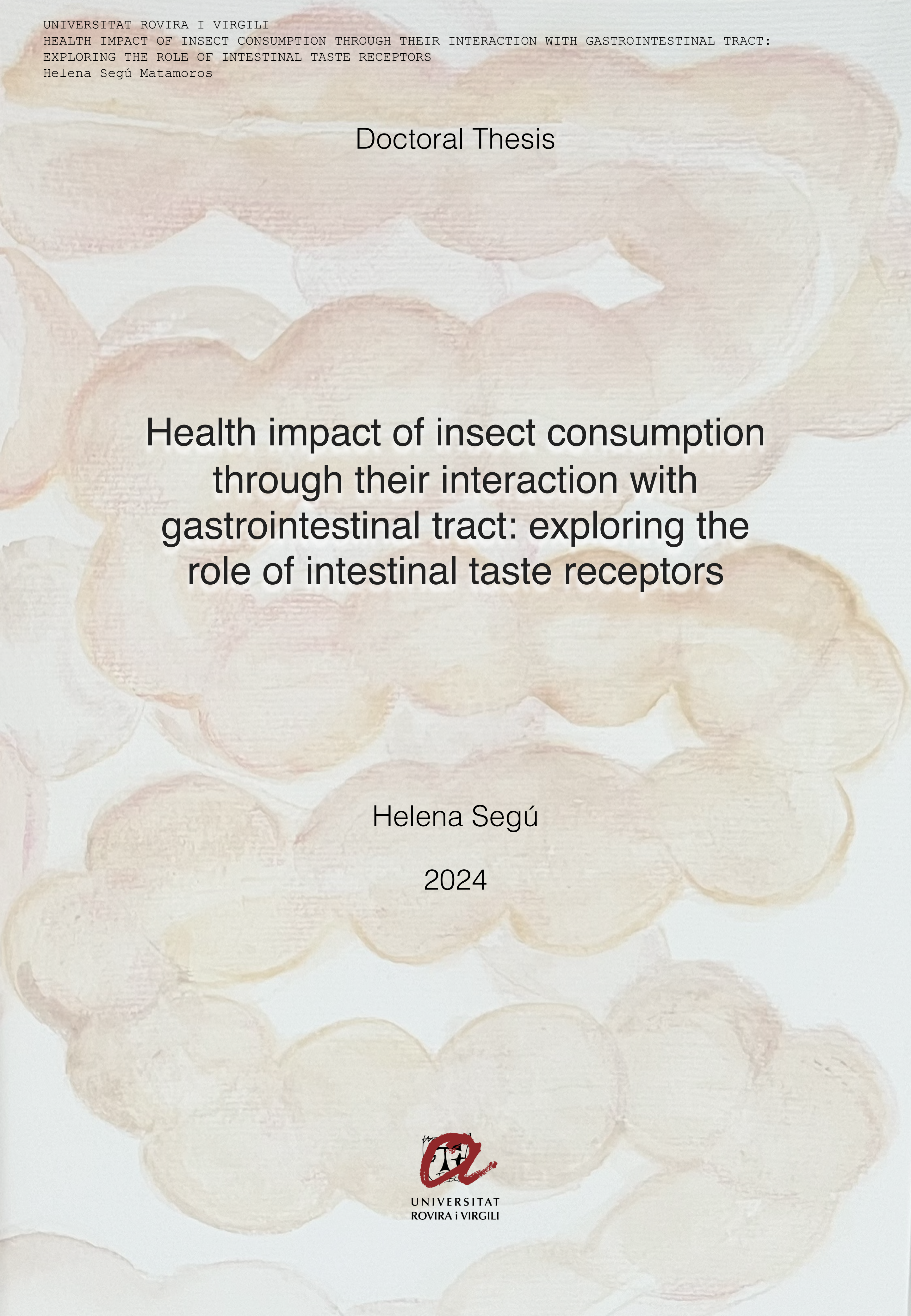

Doctoral Thesis

# Health impact of insect consumption through their interaction with gastrointestinal tract: exploring the role of intestinal taste receptors

Helena Segú

2024

UNIVERSITAT
ROVIRA i VIRGILI

Helena Segú

# Health impact of insect consumption through their interaction with gastrointestinal tract: exploring the role of intestinal taste receptors

Doctoral Thesis

Supervised by Dr. Maria Teresa Blay Olivé and
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Biochemistry & Biotechnology Department
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Tarragona, 2024

UNIVERSITAT
ROVIRA i VIRGILI

FAIG CONSTAR que aquest treball, titulat "**Health impact of insect consumption through their interaction with gastrointestinal tract: exploring the role of intestinal taste receptors**", que presenta **Helena Segú** per a l'obtenció del títol de Doctor, ha estat realitzat sota la meva direcció al Departament de Bioquímica i Biotecnologia d'aquesta universitat.

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HAGO CONSTAR que el presente trabajo, titulado "**Health impact of insect consumption through their interaction with gastrointestinal tract: exploring the role of intestinal taste receptors**", que presenta **Helena Segú** para la obtención del título de Doctor, ha sido realizado bajo mi dirección en el Departamento de Bioquímica y Biotecnología de esta universidad.

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I STATE that the present study, entitled "**Health impact of insect consumption through their interaction with gastrointestinal tract: exploring the role of intestinal taste receptors**", presented by **Helena Segú** for the award of the degree of Doctor, has been carried out under my supervision at the Department of Biochemistry and Biotechnology of this university.

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A l'avi, l'àvia i la mama

“Que tot està per fer i tot és possible.”

Miquel Martí i Pol

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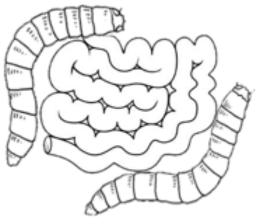
# SUMMARIES



# SUMMARY

The consumption of insects is gaining attention due to its perceived sustainability and health benefits. Compared to traditional protein sources, insect farming is more environmentally friendly, utilising organic waste, emitting fewer greenhouse gases, and requiring less water. Moreover, insects provide a high nutritional value, including essential amino acids and micronutrients, while also containing bioactive compounds like peptides and antioxidants. These compounds show promise in reducing chronic disease risk, improving immune function, and promoting intestinal health. Nevertheless, further research is required to elucidate the health implications of insect consumption and to enhance social acceptance. In this sense, the intestinal tract's health is vital for overall well-being, as it serves as the body's first barrier against food and pathogens. Additionally, the presence of taste receptors in the intestine, which are known to have physiological effects beyond their role in taste perception, highlights the potential for dietary choices to influence intestinal and overall health through these receptors.

In this context, the current thesis examines the impact of insect consumption on intestinal health and, subsequently, on systemic responses, in both healthy and induced inflammation conditions in rats. Furthermore, it investigates the potential modulation of intestinal taste receptors by insects and its health implications.

The results demonstrate that the administration of *Tenebrio molitor* and *Alphitobius diaperinus* to normal chow-feed Wistar rats have no adverse health effects. In fact, supplementation with insects improve alterations in uraemia, glycaemia, and intestinal surface area induced by LPS injection. Insect supplementation modulates umami and bitter taste receptor expression, primarily in the colon, and it has implications for metabolic and inflammatory responses. On the other hand, consumption of *Alphitobius diaperinus*, as a unique source of protein in the diet, resulted in a less pro-inflammatory profile compared to conventional protein sources and induced changes in metabolic status in healthy rats. Additionally, consumption of this insect-based diet attenuates some alterations associated with an obesogenic status, ameliorating inflammatory and metabolic disturbances.

In conclusion, the present thesis demonstrates favourable effects of insect consumption intestinal health as well as inflammatory and metabolic amelioration when insects are consumed chronically in both healthy and pathological conditions. Furthermore, the results reinforce the idea that the expression of intestinal taste receptors is highly susceptible to changes in dietary patterns and health status and suggest its involvement in several important functions including immune and metabolic processes.

# RESUM

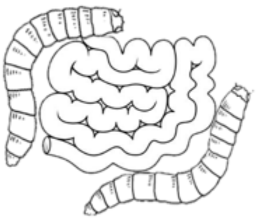
El consum d'insectes està guanyant atenció a causa de la percepció de sostenibilitat i beneficis per a la salut. En comparació amb les fonts tradicionals de proteïnes, el cultiu d'insectes és més respectuós amb el medi ambient, utilitzant residus orgànics, emetent menys gasos d'efecte hivernacle i requerint menys aigua. A més, els insectes aporten un alt valor nutricional, incloent aminoàcids essencials i micronutrients, alhora que contenen compostos bioactius com pèptids i antioxidants. Aquests compostos són prometedors per reduir el risc de malalties cròniques, millorar la funció immunitària i promoure la salut intestinal. No obstant això, es requereixen més estudis per dilucidar les implicacions que té el consum d’insectes per a la salut i millorar-ne l'acceptació social. En aquest sentit, la salut del tracte intestinal és vital per al benestar general, ja que serveix com a primera barrera de l'organisme contra aliments i patògens. A més, la presència de receptors del gust a l'intestí, que se sap que tenen efectes fisiològics més enllà del seu paper en la percepció del gust, posa de manifest el potencial de les tries dietètiques per influir en la salut intestinal i general a través d'aquests receptors.

En aquest context, la tesi actual examina l'impacte del consum d'insectes en la salut intestinal i, subseqüentment, en les respostes sistèmiques, tant en condicions de salut com d’inflamació induïda en rates. A més, investiga la possible modulació dels receptors del gust intestinal per insectes i les seves implicacions per a la salut.

Els resultats demostren que l’administració de *Tenebrio molitor* i *Alphitobius diaperinus* en rates Wistar amb dieta estàndard no té efectes adversos sobre la salut. De fet, la suplementació amb insectes millora alteracions en la urèmia, la glucèmia i la superfície intestinal induïdes per la injecció de LPS. La suplementació amb insectes modula l'expressió del receptor del gust umami i amarg, principalment al còlon, i té implicacions en les respostes metabòliques i inflamatòries. D'altra banda, el consum d'*Alphitobius diaperinus*, com a font única de proteïnes de la dieta, resulta en un perfil menys proinflamatori en comparació amb les fonts de proteïnes convencionals i va induir canvis en l'estat metabòlic en rates sanes. A més, el consum d'aquesta dieta basada en insectes atenua algunes alteracions associades a un estat d’obesitat, millorant-ne l’estat inflamatori i metabòlic.

En conclusió, la present tesi demostra efectes favorables del consum d'insectes en la salut intestinal, així com la millora inflamatòria i metabòlica quan els insectes es consumeixen de manera crònica tant en condicions saludables com patològiques. A més, els resultats reforcen la idea que l'expressió dels receptors intestinals del gust és altament susceptible a canvis en els patrons dietètics i l'estat de salut, i suggereixen la seva implicació en diverses funcions importants, inclosos els processos immunitaris i metabòlics.

# LIST OF ABBREVIATIONS



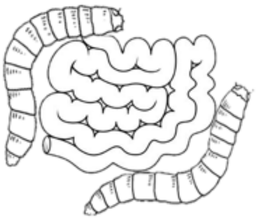
# LIST OF ABBREVIATIONS

| | |
|---|---|
| AA | amino acids |
| Ag | antigens |
| AMP | antimicrobial peptides |
| ATP | adenosine-5'-triphosphate |
| BCAAs | branched-chain amino acids |
| BW | body weight |
| cAMP | cyclic adenosine monophosphate |
| CAF | cafeteria diet |
| CCK | cholecystokinin |
| CVD | cardiovascular diseases |
| DC | dendritic cells |
| DSS | dextran sodium sulphate |
| EAA | essential amino acid |
| EFSA | European Food Safety Authority |
| FAO | Food and Agriculture Organization of the United Nations |
| GALT | gut-associated lymphoid tissue |
| GHG | greenhouse gas |
| GI | gastrointestinal tract |
| GLP-1 | glucagon-like peptide 1 |
| GPCR | G-protein-coupled receptor |
| HDM | house dust mites |
| HFD | high-fat diet |

| | |
|---|---|
| IBD | inflammatory bowel disease |
| Ig | immunoglobulin |
| IL | interleukin |
| ILCs | innate lymphoid cells |
| IP3 | inositol-1,4,5-triphosphate |
| LP | *lamina propria* |
| LPS | lipopolysaccharide |
| M | absorptive surface area |
| M cells | microfold cells |
| MACs | microbiota-accessible carbohydrates |
| MLN | mesenteric lymphatic node |
| MPO | myeloperoxidase |
| MSG | monosodium glutamate |
| MUC | mucin |
| MUFAs | monounsaturated fatty acids |
| NEFAs | non-essential fatty acids |
| OVA | ovalbumin |
| PPIA | cyclophilin-E |
| PUFAs | polyunsaturated fatty acids |
| ROS | reactive oxygen species |
| SCFAs | short-chain fatty acids |
| SCP | “single” cell protein |
| SFAs | saturated fatty acids |

| | |
|---|---|
| **sIgA** | secretory immunoglobulin A |
| **TAG** | triglycerides |
| **TASR** | taste receptor |
| **TGF- β** | transforming growth factor β |
| **TJ** | tight junction |
| **TLR** | toll-like receptors |
| **TNF- α** | tumor necrosis factor α |
| **Tregs** | regulatory T cells |

# INTRODUCTION



# INTRODUCTION

## 1. Opening

The search for sustainable protein sources that not only contribute to reducing the environmental impact of food production but also could also benefit our health is a global goal. In this context, insects have been postulated as a high-quality protein source, apporting some nutrients apart from protein, as well as its production is considered environmentally friendly [1]. They are supposed to be a good alternative for the conventional sources of protein, but there are some barriers to their social acceptance. In this sense, further studies demonstrating the effects of insect consumption on health, as well as elucidating its benefits, are crucial to increase its acceptance [2].

Dietary components are one of the most important factors influencing health. In this sense, the intestinal barrier serves as the first line of defence against luminal antigens, pathogens and toxins, while facilitating nutrient absorption and immune surveillance [3]. Disruption of intestinal barrier integrity, whether due to dietary factors, dysbiosis or immune dysregulation, can have consequences for systemic health. For example, in obesity, excessive consumption of high-fat, high-sugar diets can disrupt intestinal barrier integrity and promote low-grade inflammation, contributing to intestinal dysfunction and systemic metabolic endotoxemia [4]. However, a balanced diet rich in fibre and bioactive compounds is known to promote and maintain healthy intestinal function [5].

In terms of food recognition, taste receptors have been identified as potential targets of food components. These receptors are found in several extra-oral tissues, including the gut, and are associated with sensing luminal contents and modulating epithelial responses, which have been described to regulate enteroendocrine and immune function [6]. Moreover, different stimuli such as food components, pathology, or age, could modulate the expression of taste receptors and influence their physiological functions [7].

In light of this evidence, exploring the relationship between diet, intestinal health, and overall wellbeing is a compelling avenue for research. Therefore, the aim of this introduction is to review the current knowledge on (1) the sustainability of insect production, the benefits and concerns of insect consumption and its possible impact on the maintenance of intestinal function; (2) the structure of the gastrointestinal tract, the function of the intestinal barrier and its modulation; as well as (3) the function of taste receptors, the intestinal and immune implications of their activation, and their possible modulation by diet.

## 2. Insects as an alternative source of protein

### 2.1. The need to find alternative sources of protein

The global population is expanding due to a combination of factors, including the decline in mortality rates globally, elevated fertility rates in specific regions, and the phenomenon of population momentum. Consequently, projections indicate an increase of 1.3 billion people over the next 30 years, culminating in an estimated 9.7 billion by 2050 [8]. Therefore, the food production needs to increase, and a pivotal concern in this context is the escalating demand for protein, anticipated to rise approximately 70% between 2012 and 2050 [1].

Among the spectrum of essential macro and micronutrients derived from the diet, protein assumes a critical role in tissue formation, repair processes, and the constitution of the enzymes and hormones [9,10]. Adequate protein intake represents the 10% of energy intake and its quality is intricately linked to the presence of essential amino acids, with animal sources traditionally recognized for their superior quality compared to most of plants sources [11]. Western dietary patterns, characterized by elevated consumption of dairy products, meat, and eggs; often exceeds recommended dietary thresholds for saturated fats and red meat [12]. Prolonged adherence to such diets correlates significantly with heightened mortality rates [11]. Nevertheless, eggs, dairy products, meat, fish and their derivatives, when consumed in adequate quantities, stand out as conventional sources of protein and also contain other essential nutrients such as lipids, vitamins and iron. This positions them as highly nutritious options, particularly in regions where access to food is limited, and protects against malnutrition [13]. Hence, their production has experimented exponential growth to assess this great demand for high quality protein, and it will continue to increase, mainly by large livestock production.

Nowadays, it is well known that livestock animals contribute around 15% of total greenhouse gas (GHG) emissions [14], mainly from cattle and sheep production, both directly (through methane production by ruminants) and indirectly (through reactive nitrogen and carbon dioxide emissions and deforestation associated with pasture and fodder crop production) [15]. In addition, water pollution and scarcity are also linked to this animal food production, positioning it as a major contributor to climate change [16].

Thus, the big challenge is to feed an expanding human population with environmentally friendly food production approaches [14,17]. In this sense, the conventional methods of food production are being re-evaluated in the face of this challenge, leading to a growing interest in agroecology. This approach aims to transform current production systems to enhance their sustainability and reduce their environmental footprint [18]. Another aspect of this transition involves reducing the consumption of meat and dairy products. Numerous studies have emphasized the importance of this dietary shift in achieving climate change targets [19]. As mentioned before, the livestock sector is a significant contributor to greenhouse gas emissions, and reducing its scale could have a substantial impact on mitigating climate change. Nevertheless, in the quest for global nutritional adequacy, there is a burgeoning interest in exploring sustainable methods to produce novel and high-quality protein sources, that can serve both as human food and as ingredients for animal feed. This includes exploring currently underutilized plant sources, insects, and single-celled organisms, like bacteria, algae and fungi [16], as well as *in vitro* cultured "meat" [20]. These alternatives offer the possibility of diversifying or partially replacing traditional meat sources, in line with the objectives of sustainability and reducing environmental impact.

### 2.2. Environmental impact of different protein sources

Some interesting reviews have focused on the environmental benefits of dietary protein sources that represent alternatives to meat [21–24]. Ruminant meats, for example, have impacts that are 3–10 times those of other animal-based foods and 20–100 times those of plant-based foods for all indicators examined [25].

In this context, vegetable sources seem to be an accepted substitute, since they are naturally present in people's diets, bring health and environmental benefits and have lower production associated costs. Vegetable protein source includes leguminous plants (e.g., soy, lentils, lupin, chickpeas), oil plants, cereals (e.g., maize, rice, wheat) and pseudo cereals (e.g., amaranth and quinoa) [24,26]. The studies demonstrated that the production of 1kg of beef requires 50 times more land and about 15 times more water than the production of 1kg of grain and vegetables. Moreover, replacing 25–50% of animal-derived foods with plant-based foods has been reported to result in a 40% reduction in nitrogen emissions and 25–40% reduction in others GHG emissions in the European Union [12]. Even though plant-based source has also a beneficial effect on human health, reducing risks of some chronic diseases, they are generally deficient in essential amino acids [27]. So, combining plant sources in the right balance is a good solution to achieve adequate essential amino acid profiles. On the other hand, microbial protein, or "single" cell protein (SCP), is established as a potential alternative due to microorganism cultivation does not require a large number of lands as in crops and animal husbandry, since microorganisms are usually grown in tanks or reactors. SCP includes the protein derived from unicellular or even multicellular microorganisms, mainly fungi (yeasts and filamentous fungi), microalgae (cyanobacteria and unicellular eukaryotes) and bacteria [22].

Finally, although insects are among the most controversial alternatives due to conflicts with the cultural habits of various populations, they are present in the diets of some populations around the world and have numerous environmental and nutritional benefits. Studies indicate that environmental indicators such as land use, water footprint, and GHG emissions are 40–60% lower for the feed and food of edible insects compared to traditional livestock [1,28,29]. In addition, the ability of insects to feed on bio-waste, such as mushroom waste or fruit peels, further reduces environmental impact by turning waste into compost, which also provides new strategies for a circular bioeconomy [30,31]. Moreover, the feed conversion efficiency, which means the feed needed to produce 1kg of increase in body weight, significantly differs between conventional livestock and insects. For instance, crickets are more efficient in converting feed into body mass compared to traditional livestock. The feed conversion ratio of edible weight shows that crickets are twice as efficient as chickens, four times more than pigs, and twelve times more than cattle [32], suggesting that insects are cost-effective alternative source. This efficiency underscores insects' role as a sustainable food system, which, besides their nutritional value, contributes positively to ecological, environmental, and economic impacts [33].

Therefore, in terms of environmental impact, these alternative protein sources collectively offer a more sustainable solution than traditional livestock farming. They reduce the strain on land and water resources and contribute to lower greenhouse gas emissions. This shift towards alternative proteins is essential for mitigating the environmental impacts of food production and supporting global food security. In particular, the high feed conversion efficiency, short life cycles, high reproduction rates [34], and the ability to thrive on a wide range of foods, including by-products from food processing and high-impact waste streams [35], make insects one of the most environmentally beneficial and economically viable options [36].

### 2.3. Insect consumption by humans, entomophagy

Insects have been consumed since early in human evolution and more than 2000 different insect species are consumed on a regular basis by two billion people worldwide, principally in Africa, Asia, Oceania, and South America [26,37,38]. This practice of eat insects is known as entomophagy [39], and it includes the consumption of beetles (Coleoptera), caterpillars (larvae of butterflies and moths; Lepidoptera), bees, wasps, and ants (Hymenoptera), and locusts, grasshoppers, and crickets (Orthoptera) [32]. For millennia, insects have been an important source of dietary protein in some human populations [40]. However, in western countries entomophagy is uncommon, and people associate it to a disgusting practice [41,42]. Due to this cultural barrier, the use of processed insects as powder ingredient, which is then incorporated into products such as burgers, pasta, or snacks, could enhance consumers' acceptance [43]. On the other hand, more investigations that verify the benefits of insects' consumption at nutritional level and environmental advantages, could also improve the knowledge and the acceptance to introduce insects in our diets [42]. So, insect protein could become part of the total protein consumed by humans in the near future [44].

This emerging trend in the Western societies began in 2013, when the Food and Agriculture Organization of the United Nations (FAO) pointed out the need to examine modern food science practices to increase the trade, consumption, and acceptance of insects [32]. Regulation 2015/2283 of the European Parliament and the Council of the European Union, further marked a pivotal moment, incorporating whole insects and their parts in the category of novel foods [45]. Additionally, in 2015, the European Food Safety Authority (EFSA) provided a scientific opinion on insect consumption, proposing a list of insect species with high potential for use as both animal feed and human food [46]. Presently, EFSA has expressed a favourable opinion on the safety of dried yellow mealworm (*Tenebrio molitor larvae*) [47], migratory locust (*Locusta migratoria*) [48], lesser mealworm (*Alphitobius diaperinus larvae*) [49] and house cricket (*Acheta domesticus*) [50] as novel foods.

On the other hand, the European Commission has authorised the use of insects as feedstuff, initially for aquaculture farming in 2017 and subsequently for pigs and poultry in 2021 [51]. Traditionally, commercial livestock feeds rely on feed grains, such as corn, soybeans, sorghum, oats, and barley, constituting approximately one-third of all produced cereals. Insect-based feeds represent an opportunity to replace grains. Notably, there is a growing interest in incorporating insect protein into pet food formulations, with black soldier fly larvae emerging as the most widely utilized in this sector [52,53]. Furthermore, among the most promising and well-studied candidates for industrial feed production are yellow mealworms, silkworms, grasshoppers, and termites [54].

### 2.3.1. Mealworms, *Tenebrio molitor* and *Alphitobius diaperinus*, as feed and food

*Tenebrio molitor* and *Alphitobius diaperinus* are larval forms of different species of beetles from the family Tenebrionidae, known as the darkling beetles [55–58]. The female typically lays an average of 400-500 eggs, and the larval stage lasts approximately 6-8 months under optimal conditions [59]. In addition, by adjusting specific conditions, their life cycle can be managed and optimised to ensure efficient mass production [60]. As the use of insects, safety and nutritional content varies according to the life cycle stage at which they are consumed [61], it should be noted that they are mainly proposed and used in the form of raw worm derivatives or enriched with proteins (insect meals) in dry matter.

Mealworms, often found in flour and cereal grains, are also used for human consumption, with nearly two billion people worldwide consuming insects [32], and in China, the yellow mealworm is a popular dish [62]. Both of these edible larvae have been positively assessed by EFSA and have a similar nutritional profile: they are high in protein and the full spectrum of amino acids, rich in essential fatty acids and vitamins, and higher in certain minerals (particularly calcium, copper, magnesium, iron and zinc) than conventional meat and eggs [63,64].

Furthermore, mealworms have been applied for feed in different animal sectors and is one of the most studied insects. On the one hand, Bovera *et al.* 2015 demonstrated the feasibility of replacing soybean meal with *T. molitor* larvae meal in broiler diets, in terms of feed intake and growth rate, with higher feed conversion ratio and prebiotic effects in *T. molitor* consumption broilers [65]. Moreover, the same author conducted a parallel experiment during growing period of broilers, and again, demonstrated the healthiest effects of this insect when used as principal protein contributor to the diet [66]. In aquafeed, *T. molitor* has also been considered as potential alternative protein source to replace fish meal [67]. Moreover, one study described that can also be used in a bio-regenerative life support system in space, by providing an efficient system for treating plant waste and proposing it as high-quality protein for astronauts [62]. Furthermore, *T. molitor* has been shown to have a high nutritional value and considerable bioactive activity in animals and humans [29,68]. On the other hand, *A. diaperinus*, also known as buffalo mealworm, also presents high-quality protein and a well-balanced fatty acid profile [69]. However, this insect is not large used to feed animals or studied *in vivo* as yellow mealworm. In this sense, more studies could improve their application in these different sectors.

Finally, the integration of insects, and particularly, mealworms into food and feed production is in line with efforts to promote sustainability and reduce environmental impact and offers novel solutions to global food challenges. However, despite the potential for growing acceptance and regulatory support for insect consumption, there are concerns about their allergenicity, which requires further in-depth studies and regulation to ensure consumer safety.

### 2.3.2. Allergenicity to insects as food

The prevalence of food allergy in western countries is up to 6% and rising, and represents a significant health problem [70,71]. Food allergies are adverse reactions to specific food antigens, which are typically harmless to the general population but trigger immunological mechanisms in susceptible individuals [72]. In this sense, insect consumption raises concerns about allergenic responses in individuals sensitized or allergic to crustaceans, peanuts, or house dust mites (HDM) proteins [73]. Within the arthropod family, several allergens have been identified, including tropomyosin [74], arginine kinase [75] and glutathione S-transferase [76], exhibiting cross-reactivity in humans.

Indeed, Broekman *et al.* found that 13 out of 15 confirmed shrimp-allergic patients displayed allergic reactions to *T. molitor*. Exposure to yellow mealworm protein fractions revealed IgE cross-reactivity in these patients, with tropomyosin and arginine kinase identified as major cross-reactive allergens in the yellow mealworm [77]. Additionally, tropomyosin, α-amylase and muscle myosin from three edible mealworm were identified to cross-react with sera from HDM (n=11) and crustacean allergic patients (n=8) [78]. Another study conducted by the same authors introduced other insects (*Z. morio*, *A. diaperinus*, *G. mellonella*, *H. illucens, A. domesticus* and *Locusta migratoria*) to the participants and concluded a propensity for allergic reactions not only to *T. molitor* but also to other insects [79]. Tropomyosin, once again, emerged as a key cross-allergen for HDM- and shrimp-allergic patients; as well as larval cuticle proteins seemed to play a major role in the cross-reactivity of patients primarily sensitized to mealworm [80]. Moreover, tropomyosin allergen from *G. bimaculatus* has described to induce an allergic reaction in individuals with crustacean allergy [81].

Additionally, a case report from France detailed a severe food anaphylaxis induced by *T. molitor* cooked-larvae in a 31-year-old man allergic to HDM but not to crustaceans. Allergic proteins responsible for this sensitization included hexamerin, tropomyosin, α-amylase, and larval cuticle proteins A1A and A2B [82].

In contrast, in healthy rodent models, long oral exposures of Sprague-Dawley rats, to doses of 300-3000 mg/kg/day of mealworm did not significant increase allergenic biomarkers such as histamine or IgE concentrations in serum [83].

However, mitigating or even eliminating insect allergens during insect processing has been explored through methods such as thermal treatment and enzymatic hydrolysis [84,85]. Further scientific studies in this direction are ongoing and offer valuable solutions to reduce the allergenicity of formulations incorporating insect proteins.

### 2.4. Nutritional value of insects

Edible insects are characterized by an excellent nutritional profile, positioning them as a viable alternative protein source for both humans and animals.

At the larval stage, insects have high percentage of protein and, in some species, reach up to 60% of total nutrients in dry weigh terms (Table 1) [86]. Moreover, insect protein contains all the essential amino acids (EAAs) and have been described to apport higher levels of them than the daily recommended for adults [87]. Beyond their protein content, edible insects exhibit elevated levels of unsaturated fatty acids, including both monounsaturated (MUFA) and polyunsaturated (PUFA) fats, as well as rich deposits of minerals, vitamins, and fibre [88,89]. Their nutritional richness extends to various micronutrients such as copper, iron, magnesium, manganese, phosphorous, selenium, and zinc, along with essential vitamins like riboflavin, pantothenic acid, biotin, and, in some cases, folic acid, positioning insects as an excellent source of diverse nutrients [90]. Furthermore, recent studies have reported that they could provide bioactive compounds, such as phenolic compounds and flavonoids [91]. Notably, the nutrient composition of insects is highly influenced by their feed [61], which opens up opportunities for regulation, enrichment, and addition of specific food ingredients.

**Table 1.** Nutritional composition of insects, *Tenebrio molitor* and *Alphitobius diaperinus*, almond (as vegetable protein source), and beef (conventional protein source) [47,92,93]. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: poly-unsaturated fatty acids.

| Nutritional value (100g of product) | *Tenebrio molitor* | *Alphitobius diaperinus* | Almond | Beef |
|---|---|---|---|---|
| Energy (kcal) | 505 | 510 | 579 | 198 |
| Protein (g) | **57.2** | **59.6** | **21.2** | **19.4** |
| Total Fat (g) | 28.4 | 28.7 | 49.9 | 12.7 |
| SFA (g) | 6.9 | 10.1 | 3.5 | 5.3 |
| MUFA (g) | 14.4 | 9.7 | 31.6 | 4.8 |
| PUFA (g) | 7.0 | 8.8 | 12.3 | 0.5 |
| Carbohydrates (g) | 1.8 | 2.7 | 21.6 | 0.0 |
| Sugars (g) | 0.2 | 0.5 | 3.6 | 0.0 |
| Fibre (g) | 6.4 | 7.74 | 12.5 | 0.0 |

Conventional animal-based foods (eggs, milk, and meat), as well as plant-based protein-dense food (soy) are considered high-quality protein sources because they meet all the current essential or indispensable amino-acid requirements and are easily digested and absorbed by mammals small intestine [94]. In contrast, several plant-based sources of protein (cereals, beans, legumes, or vegetables) lack some EAAs, and protein digestibility is lower than in the edible insects [95]. When insects are compared with all these protein sources, studies suggest that the protein and EAA content of insects is comparable to conventional high-quality animal and plant foods [96], and they present higher energy value, unsaturated fatty acids, and certain vitamins, but lower saturated fatty acids than meat. Moreover, contrary to meat, insects are also a source of vitamin C and dietary fibre [97]. The protein content in common edible insects such as *Acheta domesticus* (72.45%), *Tenebrio molitor* (58%) and *Antharaea assamensis pupae* (38.05%) are higher than that in legumes such as lentils (26.7%), beans (23.5%) and soybean (41.1%) [98].

Insect's digestibility is variable and affected by the presence of chitin [99], which is not degraded nor absorbed in the small intestine although higher in plant foods. Recent studies point to the need for increased and improved assessments of ileal amino-acids digestibility and postprandial protein synthesis *in vivo*, to adequately evaluate and compare the quality of insect-derived proteins with others protein sources [96]. In this sense, Lanng *et al.* described a larger amount of aspartate, methionine, glutamate, branched-chain amino acids (BCAA) and the aromatic amino acids, tyrosine and phenylalanine, that reach the small intestine, after ingestion of insect comparing to ingestion of pork [100]. Furthermore, Dai *et al.* also demonstrated a greater leucine, BCAA and EAA levels after cricket protein consumption, while non-essential amino acids and total amino acids concentration was greater for beef-derived protein [101].

Considering the existing evidence, insects have been postulated as a potential source of high-quality protein, in terms of essential amino acid content and digestibility. Thus, insects are among the suggested future foods to provide good-quality alternatives to current protein source foods [26].

### 2.4.1. Health beyond nutrition: bioactivity of edible insects

As mentioned above, insects could be a source of bioactive compounds, which can have a beneficial effect on health when consumed. Examples of these compounds found in insects are peptides, phenolic compounds, chitin, and chitosan [102–105]. Bioactive peptides consist of 2 to 20 amino acids as a non-active sequence encoded in parent proteins, obtained from enzymatic hydrolysis, in vivo or in vitro digestion, microbial fermentation, and other processing technologies [106,107]. Chitin and its derivative, chitosan, are high present polysaccharides in insects, because is the main organic skeleton material of the arthropod exoskeleton [102]. For his part, large number of polyphenols identified in insects are often synthesized by host plants before being metabolized or absorbed by the insects themselves [91]. The bioactivity of these compounds has been demonstrated to generate various health benefits.

For all these reasons, there is a growing interest in the potential bioactive properties of insect components and their protein hydrolysates [29,44], including antioxidant, antimicrobial, antihypertensive and anti-diabetic properties, which could be applied in functional foods or nutraceuticals [98].

#### Effects on metabolism and food intake

Bioactivity of insects has been suggested in terms of amelioration of metabolism and weight control. On one hand, some studies demonstrated the inhibition of DPP-IV, an enzyme responsible of the reduction of insulin and, consequently, the increase postprandial blood glucose, by *Gryllodes sigillatus* or *Antheraea assamensis* hydrolysates, suggesting antidiabetic properties [108,109]. Moreover, *in vivo* studies reported the effectiveness of silkworm ethanolic extracts and cricket glycosaminoglycans to improve the glucose metabolism and anti-oxidative effect in type-2 diabetic mice [110,111]. Chronic supplementation with *Proteatia brevitaris* larvae for seven weeks improves the health status of mice fed a high-fat diet, by reducing body weight (BW) gain, epididymal and subcutaneous fat weight, lipid accumulation in liver and plasmatic levels [112]. Another study with ethanolic extracts from *Gryllus bimaculatus* in rats fed high-fat diet, counteract the damage induced by the diet with a reduction in abdominal and epididymal fat weight, cholesterol, and triglycerides levels, suggesting their potential as anti-atherosclerosis or inflammation [113]. However, with regard to the effects on BW, there is no consensus on the intake of *T. molitor* intake in animal models. High doses of *T. molitor* supplementation showed a tendency to increase the BW and food consumption in male rats [114]. In obese rats, the incorporation of this insect in the diet caused a lipid-lowering effects in liver and plasma [115]. On the contrary, other authors reported no effect in BW or food intake after chronic treatments in male [116] or female rats [83]; while others indicated that mealworm-based diets led to reduced weight gain and improved metabolism in diet-induced obesity mice [58]. Moreover, in a previous study of our research group, *A. diaperinus* acutely modified intestinal enterohormone secretion *ex vivo* and it was associated to an increase of food intake [117].

### Antihypertensive effects

Antihypertensive properties are principally suggested to achieve through the inhibition of angiotensin I-converting enzyme [118]. Sousa *et al.* demonstrated the ability of protein hydrolysates from *Alphitobius diaperinus* to effectively inhibit this enzyme [119]. *In vivo*, *Tenebrio molitor* were described to reduce blood pressure, heart rate and coronary perfusion pressure of spontaneously hypertensive rats [116].

### Antioxidant and immunomodulatory properties

The antioxidant capacity *in vitro* was determined to be 5-fold higher than fresh orange juice, for different extracts of grasshoppers, silkworm, and crickets [120]. Zielinska *et al.* demonstrated that total phenolic content and antioxidant capacity, measured as the ability to neutralize ABTS·+ and DPPH·, increased when the percentage of insect flour added in muffins also increase [121]. This property was associated to the phenolic content but also to peptides from protein hydrolysates [118]. An *in silico* analysis proposed that some peptides with anti-inflammatory activity could be found in *T. molitor* [122]. In this sense, another work demonstrated that *in vitro* digested protein preparations of insects (*G. sigillatus*, *T. molitor*, and *S. gragaria*) could be sources of bioactive peptides with antioxidant and anti-inflammatory effects, being accentuated after heat treatment process, that significantly improves these properties [123]. The study also showed that protein hydrolysates exert anti-inflammatory effect by the inhibition of lipoxygenases (LOX) and cyclooxygenases (COX). Although this property of insects has not been widely researched, a study reported that 14-days cricket consumption in healthy adults, reduced systemic inflammation by decreasing plasmatic TNF-$\alpha$ levels, and improved gut health by increasing the growth of probiotic bacterium [124]. Moreover, Park *et al.* reported the protective effect of bioactive constituents of *A. dichotoma* in the vascular barrier integrity and inflammatory responses on lipopolysaccharide (LPS)-induced vascular inflammation [125]. Furthermore, antimicrobial peptides such as defensins, cecropins, and melittin, that are essential components of the innate immune system, could be obtained from some insect's species [126]. As well, chitosan present in insects could act as a chelating agent in biological systems and has antimicrobial activity against bacteria, yeasts, and fungi [127].

### Effects on intestinal health

A few studies have also looked specifically at the effects of insect consumption on the intestinal tract. For instance, *G. bimaculatus* was found to suppress intestinal oxidative stress and mitigate inflammatory responses in the small intestine, protecting it from alcohol-induced hyperpermeability in mice after alcohol exposure [128]. Another study reported the reduction of IL-1β, IL-6, and TNF-α gene expression, as well as the attenuation of pathological changes in the colon of DSS-induced mice when fed *Tenebrio molitor* larvae powder [129].

In a recent work, the partially and total substitution of fishmeal with *A. diaperinus* and *T. molitor* meals, showed a healthy growth, healthy microbiome composition and healthy immune gene expression in Atlantic salmons [130]. Furthermore, Ge *et al.* proposed that a diet incorporating yellow mealworm improves the intestinal immune function in an intestinal damage model of fish, providing evidence of health benefits also in the aquaculture industry [131].

On the other hand, considering that the intestine is a habitat for thousands of bacteria that make up the microbiota, which can metabolize compounds from ingested food and influence both intestinal and overall health, some studies have explored the modulation of microbiota by insect consumption. For example, Young *et al.* studied the fermentation of different *in vitro*-digested larvae in human faecal batch cultures. They demonstrated the increase of bacteria with anti-inflammatory properties and associations with gut health [132]. Additionally, another study with insect larvae were found to induce a slight improvement in the caecal microbiota of chicken by enhancing the population of short-chain fatty acids (SCFA)-producing bacteria [133]. In rats, a partially substitution of meat by protein isolated from *A. diaperinus* also results in alterations of the microbiome composition, accompanied by gastrointestinal and plasmatic changes of metabolite profile [100]. Moreover, in the study of Kang *et al. T. molitor* or *A. diaperinus* were included in the diet of diet-induced obesity mice and resulted in intestinal microbiome changes and metabolic benefits for these animals [58]. According to all these evidence, consumption of insects could induce changes in the microbiome of animals and humans and, consequently, in intestinal and systemic system, that could be associated to health benefits.

**Table 2**. Publications on the effects of insect consumption in animal models on inflammation, intestinal health and barrier function.

| Insect specie | Cell type or animal model | Health status | Effect | Reference |
|---|---|---|---|---|
| *A. dichotoma* | *In vitro* | LPS-treated endothelial cells | Protective inflammation and barrier disruption | Park *et al.* 2020 [125] |
| *G. sigillatus,*<br>*T. molitor,*<br>*S. gragaria* | *In vitro* | - | Inhibition of LOX and COX | Zielińska *et al.* 2018 [123] |
| *G. sigillatus* | Human | Healthy | ↑ Growth probiotic bacterium<br>↓ TNF- α plasmatic levels | Stull *et al.* 2018 [124] |
| *T. molitor* | Mice | DSS-induced colitis | Colonic protection<br>↓ IL-1β, IL-6, TNF-α gene expression | Park *et al.* 2023 [129] |
| *G. bimaculatus* | Mice | Alcohol- induced hyperpermeability and oxidative stress | ↓ Oxidative stress<br>↓ Inflammation | Hwang *et al.* 2019 [128] |
| *T. molitor*<br>*A. diaperinus* | Mice | HFD- induced obesity | ↓ Immune related gene expression and apoptosis | Kang *et al.* 2023 [58] |
| *T. molitor* | Largemouth bass | Intestinal damage | Improve intestinal immune function | Ge *et al.* 2023 [131] |
| *T. molitor*<br>*A. diaperinus* | Atlantic salmon | Healthy | ↑ Intestinal immune gene expression | Habte-Tsion *et al.* 2024 [130] |
| *T. commodus*<br>*C. giveni* | Human faecal batch cultures | Healthy | Improve microbiota<br>↑ Anti-inflammatory properties and gut health | Young *et al.* 2020 [132] |
| *H. illucens*<br>*T. molitor* | Broiler chickens | Healthy | = Mucin composition<br>= Local immune response<br>Improve caecal microbiota | Colombino *et al.* 2021 [133] |
| *T. molitor* | Chicken | Healthy | ↑ Feed conversion ratio<br>↑ Length of intestine | Bovera *et al.* 2016 [66] |

Given all these evidence, insects or products incorporating them as ingredients have been depicted as comparably healthy to meat products [89], and potential sources of bioactive compounds [91]. Nevertheless, a gap exists in our understanding of the intestinal-level bioactivity of insects. Thus, conducting more *in vivo* studies that specifically explore their health benefits could serve as a motivating factor for people to include this alternative protein source as a regular component of their daily diet.

Exploring the effects of insect consumption on the gastrointestinal tract is a promising area of research to understand its wider impact on overall health. In addition, long-term studies with different proportions of insects and insect proteins are needed to fully elucidate their effects and underlying mechanisms in the body [1]. This research is essential to increase the scientific knowledge of these novel foods in order to increase the social acceptability of edible insects, the main barrier to their introduction into Western diets.

## 3. The gastrointestinal tract and its functions' modulation by food

### 3.1. Anatomy, physiology, and general function of intestine

The gastrointestinal (GI) tract represents the largest surface between the external environment and the human body [134]. The intestine, also called bowel or colloquially "gut", is the organ responsible of the digestion and absorption of nutrients. When the food components reach the intestine, it concludes the digestive process initiated in the oral cavity. Firstly, in the small intestine, water, and small-molecules nutrients, including sugars, free amino acids, and ions, are absorbed into the blood circulation. Subsequently, in the large intestine or colon, larger molecules and fibre are accumulated to be breakdown by microbiota, also known as fibre fermentation, and absorption of other nutrients, such as vitamins, takes place [135]. Moreover, the intestinal epithelium serves as a physical barrier that separates two opposing compartments. In this sense, beyond its role in nutrient absorption, it assumes the role in the body's defence against luminal pathogens [136], as well as it controls the mucosal immune response and metabolic activity of the intestine.

The GI system is connected to the vascular and lymphatic systems, facilitating the regulation of food intake and digestive function, and the delivery of ingested compounds to organs throughout the body [137,138]. Moreover, it is really connected with the nervous system. In the wall of the GI tract, an extensive neuronal network forms the enteric nervous system (ENS), which is also involved in the motility, secretory and immunological activity of the GI tract [139]. Furthermore, a gut-brain axis has also been described, whereby various peptides or hormones that are secreted by the intestine in response to nutrients enter the circulation and act directly on the brain to help maintain homeostasis [138]. These links with the circulatory, immune and nervous systems underline the systemic impact of intestinal processes, extending their influence far beyond the confines of the digestive tract.

In recent years, the intestine has gained attention for its pivotal role in the pathophysiology of some health disorders. Moreover, the existing knowledge about the cellular effects exerted by nutrients through the intestine supports the possibility of the deregulation of intestinal homeostasis by some dietary components, while others may offer a positive modulation [140,141]. Thus, the identification and interpretation of the mechanisms by which nutritional habits and dietary compounds influence intestinal health by promoting and/or restoring its homeostasis is an important field of study which requires an understanding of intestinal structure and its functions.

### Intestinal structure and digestion process

The human adult intestinal system consists of two parts: the small intestine, measuring approximately 6 – 7 m in length and 2.5 - 3.0 cm in diameter, and the large intestine, which ranges from 1.5 to 2 m of length with a diameter of 6 - 7.5 cm [137,142]. The small intestine is anatomically divided into three distinct regions - the duodenum, the jejunum, and the ileum-, while the large intestine is segmented in caecum, ascending and descending colon, rectum, and anus.

Histologically, the wall of the muscular tube that is recognised as intestine is formed by different layers: mucosa, submucosa, *muscularis propria* and serosa. The mucosa, the innermost layer of the intestine, is constituted by the *lamina propria* (LP), muscularis mucosae and epithelium. The *lamina propria* is a layer of reticular connective tissue formed by elastin, reticulin, collagen fibres, lymphocytes, plasma cells, granulocytes, lymphatics, and capillaries. In contrast, the *muscularis mucosae* is a thin layer of smooth muscle between the mucosa and submucosa. Both layers offer support and articulate the epithelium layer [143]. Finally, the serosa is a smooth membrane consisting of a thin layer of connective tissue and a thin layer of cells that secrete serous fluid to lubricate internal structures [144].

Along the intestine, epithelium is folded to form villi and crypts structures (Figure 1), described as luminal projections or invaginations that significantly increase the surface area [145]. Moreover, the apical surface of the enterocytes is covered by microscopic finger-like projections called microvilli (100nm in diameter), increasing more the absorptive area (M), conferring about 30 m$^2$ of surface area in the small intestine [146].

The digestive process takes place throughout the intestine, with specific functions in each part. In the duodenum, the first part of the small intestine, the digesta transition from an acidic to a neutral pH environment. Enzymes from pancreas and bile acids arrive to this region to break down proteins, fats, and carbohydrates, as well as emulsify fats [135]. Progressing from the duodenum, the jejunal and ileal parts consist in a large surface area to allow absorption of the small molecules produced. In all this process, peristalsis movements are important to exert the mixing and transporting the digesta throughout the intestinal sections. Upon reaching the colon, microbiota residing there carry out fermentation of residual carbohydrates components that have not already been digested, due to the anaerobic environment characteristic of the colon [147].

Moreover, here also occurs breakdown of remaining proteins and peptides, including those derived from the host (such as mucins). The generated products are absorbed by the enterocytes in the crypts and serve as signalling molecules, initiating systemic immune and metabolic responses [146,147]. Simultaneously, the digesta become less liquid, due to the absorption of water, also of minerals and is stored and compacted in the descending colon before eventual egestion.

### Cell types

Gastrointestinal tract is composed by different type of epithelial cells in epithelial line. At the base of the crypts are undifferentiated stem cells [135], that develop into functional epithelial cell types: enterocytes or colonocytes, Paneth cells, Tuft cells, Goblet cells and enteroendocrine cells[145] (Figure 1). These cells continuously regenerate the epithelium every 3-5 days as they migrate up the crypt-villus axis before undergoing programmed apoptosis [148]. Each type matures according to its specific function and migrates up and out of the crypt throughout the intestine; however, the distribution of each cell type varies according to the specific function of each region[149].

Intestinal epithelial cells form a physical barrier as they are impermeable to luminal contents [150]. Enterocytes, the most abundant cell type, and enteroendocrine cells are found throughout the intestine and are involved in nutrient and water absorption, and hormone production, respectively [151]. Goblet cells, found in greater numbers in large intestine, are responsible for producing mucus; while Paneth cells reside in the small intestine and contribute to innate immunity by secreting antimicrobial peptides, proteins and other components that are important in host defence and immunity [152]. Tuft cells are chemosensory cells, which have recently described also as secretory cells that initiate type 2 immune response, playing a crucial role in detecting parasitic infections or allergens and triggering the appropriate immune reaction [153]. Finally, in specific areas of the epithelium called Peyer's patches, there are specialized cells called microfold or membranous (M)-cells, that are able to transport luminal antigens and bacteria, and presenting them to lymphocytes that activate or inhibit the immune response [154].

Under epithelial cell monolayer, *lamina propria* is constituted of a very diverse populations of cells, including immune cells such as B and T lymphocytes, dendritic cells (DC), macrophages, eosinophils, neutrophils, and mast cells, among others [155]. DCs are primarily located in contact with the epithelium in the small intestine but can also be found in the large in response to inflammatory stimuli [151]. They possess the capability to capture, process, and present antigens to naïve T cells, contributing to immune response and tolerance [156]. In contrast, macrophages, extensively present throughout the entire length of the intestine, capture and kill microbes, phagocyte apoptotic material and dead cells, and secrete cytokines [157]. Neutrophils, on the other hand, play roles in both the initiation and resolution of inflammation, contributing to phagocytic activity and recruiting other immune cells through cytokine release [158]. Eosinophils, primarily located in the duodenum, are granulocytes involved in eliminating parasites, regulating T cell responses, promoting tissue repair, and contributing to allergic responses [159,160]. Mast cells, in constant interaction with eosinophils, are pivotal in the development of the allergic response by releasing histamine and inflammatory mediators, thereby influencing the functions of other immune cells [161]. Finally, T and B lymphocytes are implied in the immune adaptative response with critical roles in immune surveillance, tolerance, and defence by generating antigen-specific effector cells and antibodies to neutralize and eliminate pathogens [162].

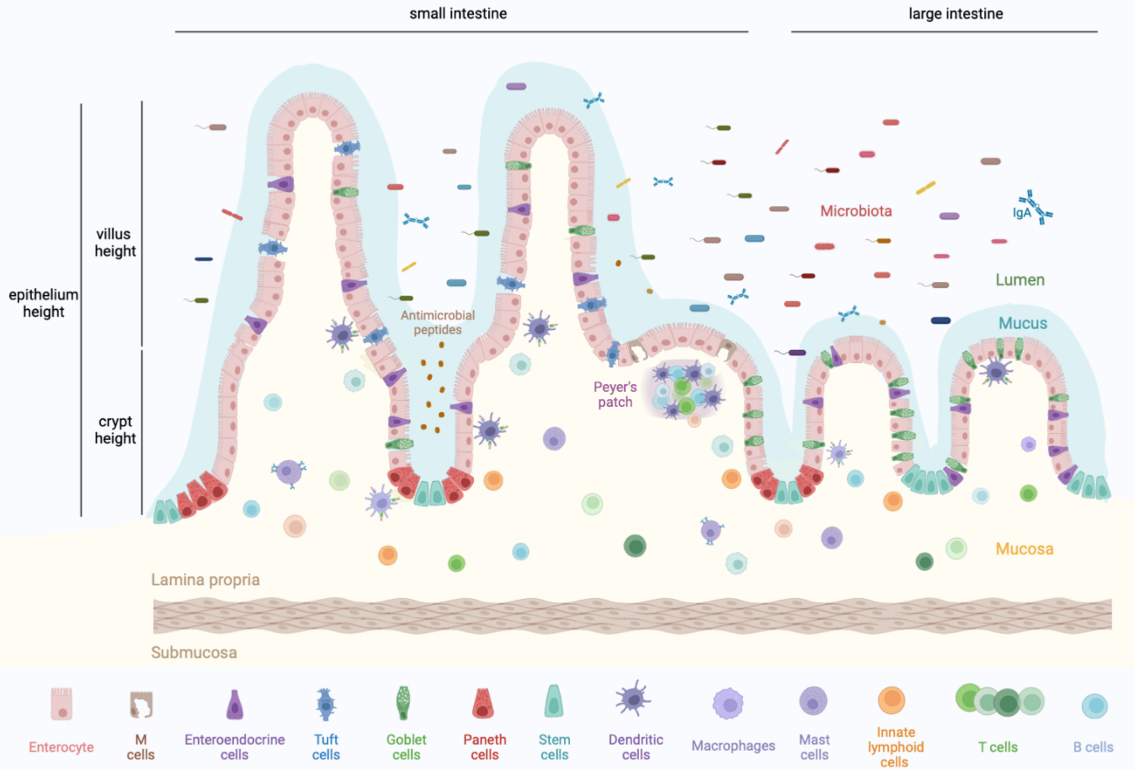


**Figure 1**. Intestinal structure and composition. Different structures of small and large intestine, and epithelium morphology. Layers of the intestinal barrier spanning from lumen to the submucosa. Within the lumen, components such as gut microbiota, antimicrobial peptides, IgA, and microbiota-derived compounds like LPS are located. The mucin layer acts as a barrier, preventing direct contact between gut microbiota and epithelial cells. The epithelial cell layer primarily consists of enterocytes, alongside other secretory cell types including, enteroendocrine, tuft cells, and goblet and Paneth cells. Various immune cell types populate the lamina propria, serving to safeguard the body against antigens and bacteria, thus preventing systemic inflammation. Created with BioRender.com.

Together, the intestinal epithelial cells and the secreted factors, along with the diverse array of immune cell present in the *lamina propria*, form the intestinal barrier [134], a concept that is explained in depth in the following section.

## 3.2. Intestinal barrier in health and disease

### 3.2.1. Homeostasis of intestinal barrier

The intestinal barrier refers to the complex system responsible for maintaining a delicate balance between absorption and protection. Comprising the microbiota, the epithelial layer, mucus, antimicrobial peptides, and immune cells, intestinal barrier acts as the first line of defence against pathogens and harmful substances present in the intestinal lumen [163]. Thus, the barrier integrated physical, biochemical, and immunological factors, a system that orchestrates a multifaceted defence mechanism to maintain intestinal and overall health.

#### Intestinal microbiota

The luminal part of the mucosa contains about 100 trillion microorganisms, known as the gut microbiota. In general, it is composed of bacteria from 6 phyla, among which *Firmicutes* and *Bacteroidetes* are the main types, but also contain fungi, viruses, phage and archaea [164]. During pregnancy, bacteria present in the placenta and the uterine environment, but mainly at birth, are considered to be the first introduction of microbiota to the child [165,166]. Then, many neonatal and postnatal factors, such as breastfeeding, also play a crucial role in establishing the microbiota and the infant's immune system [167]. Thus, the microbiota is unique between individuals and can vary within the same individual, due to age, environmental and dietary factors [168]. In addition, it varies in different anatomical parts of the GI tract, but is mainly found in the colon, where the slower flow of intestinal transit and the environment (pH, temperature and oxygen levels) favour the growth of the anaerobic types observed [168,169].

Intestinal microbiota and intestinal cells exist primarily in a symbiotic relationship, in which the bacteria contribute to the host's immunity, physiology and metabolism, while the intestine provides a nutrient-rich environment for the microbes [170]. The metabolism of polysaccharides and specific proteins could require multiple enzymes and biochemical pathways produced by the microbiota. $3.3 \times 10^6$ intestinal microbial genes have been identified, outnumbering human protein-coding genes by a factor of 150 [171]. In this sense, one of the main benefits of a 'healthy' gut microbiota is the colonic fermentation of dietary fibre, which results in the production of several metabolites, including SCFAs, ammonia, amines and phenolic compounds [172]. Most SCFAs are acetic acid, butyric acid, and propionic acid and have well-documented beneficial effects on gut and systemic health [150]. They play a crucial role in metabolic and endocrine responses, acting directly as a source of energy for host cells, but also stimulating the production of gut hormones and activating signalling to the brain to regulate food intake [170]. Additionally, the intestinal microbiota also has a potential role in maintaining the barrier integrity and function. SCFAs are also known to possess anti-inflammatory and immunomodulatory properties [172]. They enhance mucus production [173], regulate the secretion of interleukin-18 (IL-18) by epithelial and immune cells [174], and secretory immunoglobulin A (sIgA) [175], and strengthen the intestinal barrier by acting on tight junctions [176]. Furthermore, other bacterial metabolites, such as certain secondary bile acid conjugates and tryptophan metabolites, induce the development of colonic regulatory T (Treg) cells [150]. Collectively, gut metabolites play a critical role locally in maintaining intestinal homeostasis and immune regulation, while also potentially exerting systemic effects on overall health [177].

### Mucus and epithelial barrier

Mucus provides the first protection against luminal microorganisms [178], by limiting the contact of microorganisms with the epithelium, reducing the risk of invasion and infection [152]. Mucus is formed primarily of mucins secreted by Goblet cells, particularly MUC2, a high glycosylated large protein that confers a gel-forming property to mucus [179]. This dense layer also contains the antimicrobial peptides secreted by Paneth cells, that include defensins, lysozyme, chemokines, etc. [180.] Additionally, sIgA released by B cells, could attach the mucus and anchor bacteria, contributing to bacterial agglutination and facilitating their containment at the mucus layer. Furthermore, mucus provides a rich layer of carbohydrate for slow growing microbiota [181].

Following, the epithelial cells monolayer acts as the physical barrier regulating the passage of nutrients and water into the circulation, but preventing the entry of harmful substances, including pathogens, toxins, and microbiota, into the body [182]. This selective permeability is mediated by two major routes: transepithelial/transcellular and paracellular pathways. The transcellular consist in the transport of the substances predominantly regulated by selective transporters, whereas paracellular is associated with transport in the space between epithelial cells [183]. Integrity and paracellular permeability properties of epithelium are regulated by several types of adhesive structures called junctions [184]. Principally, the most apical transmembrane proteins, known as tight junction (TJ), play a crucial role in the control of cell contact and regulate the selective permeability of this layer, maintaining the intestinal homeostasis [185]. Moreover, epithelial cells include goblet and Paneth cells that are constantly fortifying the mucosal barrier through the production of mucus and AMPs, respectively; as well as M cells, known to transport the microorganisms, and macro- and soluble molecules from the intestinal lumen to the subepithelial region to initiate mucosal immune response [186].

### Immunological barrier

Below the intestinal epithelium lies the immunological barrier of the intestinal mucosa, known as the mucosal immune system, which constitutes the largest immune organ in the human body (Figure 2) [183]. Within this system, various types of immune cells are found and distributed in immune inductive and effector sites. On one hand, DC, macrophages, neutrophils, mast cells, as well as lymphoid-related cells as innate lymphoid cells (ILC), $CD4^+$ T cells (Th1, Th2, Th17, regulatory T (Treg) cells), $CD8^+$ T cells, and IgA-secreting plasma cells, diffusely existing in the *lamina propria* and conform the immune effector site. In contrast, immune inductive sites, like gut associated-lymphoid tissue (GALT), encompass organized lymphoid structures that include B cells follicles with germinal centres surrounded, by a T cell zone [186]. Together, these cells and sites, in conjunction with the other components of the barrier, work in concert in defence against pathogen infection and in the maintenance of the intestinal barrier [3,150].

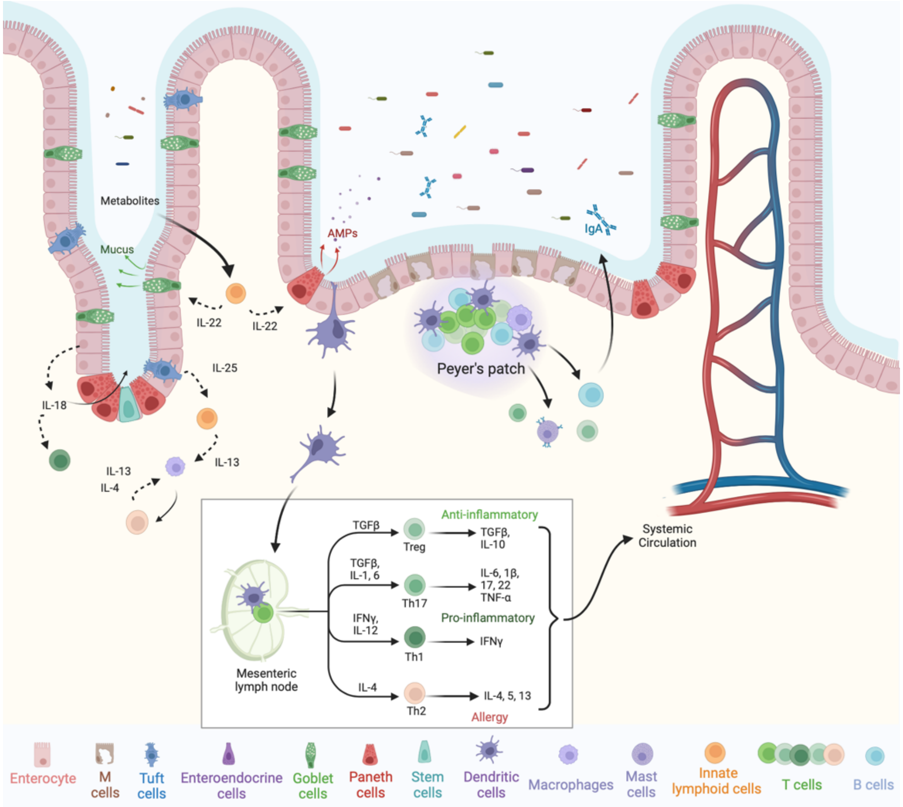


**Figure 2**. Intestinal barrier function. Activation of goblet and Paneth cells to produce mucus and AMPs respectively. IgA secretion by activation of T and B cells of Peyer's patches. DCs as cell capturing antigen and presentation in MALT structures to trigger the specific immune response. Created with BioRender.com.

Firstly, the components of innate immune system, as well as epithelial cells, employ pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), to discern between pathogenic microbial components and harmless antigens (Ags). These receptors recognize microbial-associated molecular patterns (MAMPs) that are molecular structures essential for microbial survival or damage-associated molecular patterns, released from host cells facing injury or molecular stress [187]. Their activation induces several intracellular pathways resulting in the phagocytosis and/or trigger the specific cytokine and chemokines release, and AMPs secretion, initiating a rapid proinflammatory and protective immune response [185]. Neutrophils act in the early innate immune response, phagocyting microbes and producing microbicidal factors and interleukins (ILs). Through the production of granular proteins, such as myeloperoxidase (MPO), they contribute to catalyse the formation of reactive oxygen species (ROS) that help to directly kill pathogens. Once their tasks are completed, neutrophils undergo apoptosis, and macrophages remove them to resolution inflammation and recovery of tissue function [158]. Moreover, macrophages and DCs, as mononuclear phagocytes, engulf and clear pathogenic bacteria while secreting immune mediators for cell-cell communication [177]. ILC, by its side, do not have direct cytotoxic activity but have been described to secrete IL-22 cytokine that promotes the mucus and the AMPs production [183].

Intestinal DCs and macrophages are heterogeneous and capable of generating both regulatory and effector T cell responses, in response to microbial or Ag recognition. For example, subepithelial DCs acts as immune sentinels, capturing and processing antigens and migrating to mesenteric lymphatic node (MLNs) to initiate adaptive immune response by activating naive T cells to produce (via Th1 or Th17) or supress (via Treg) inflammation [188]. On the other hand, the inductive sites sometimes exist in aggregated forms as Peyer's Patches in the small intestine and isolated lymphoid follicles (ILFs) in the small and large intestine[154]. Collectively, they form the GALT, responsible for specific immune responses to luminal antigens and immunoglobulin A (IgA) production by differentiated plasma cells [189]. GALT is formed by interfollicular area and follicle-associated epithelium, which consist in M-cells, and antigen presenting cells such as DC, that also express tight junctions and permit them to penetrate in the epithelial layer [190]. These cells take up the antigens, present them to T and B cells, and produce proinflammatory factors, such as TNF-a, IL-1β or IL-6, inducing Th17 differentiation [191]. However, in response to microbiota, macrophages also produce IL-10, while DCs produce TGF-b and retinoic acid. These compounds regulate the production of proinflammatory cytokines and the development of Tregs, which secrete more IL-10 and help to maintain immune homeostasis by suppressing excessive immune responses to harmless antigens, including commensal bacteria and dietary proteins through a mechanism known as tolerance [192]. This mechanism is crucial for preventing inappropriate inflammation in the intestine. Thus, DC and macrophages have been described to have the capacity to drive the differentiation of Treg cells that are involved in tolerance to soluble oral antigens and commensal bacteria and the ability to of provide direct signals for the differentiation of IgA-producing B cells [156].

Activation of both Th17 and Treg cells promotes class switching of B cells and secretion of secretory (s)IgA antibodies [162], in a T cell-dependent way. Subsequently, activated T and B cells migrate from GALT and MLNs into peripheral blood. Notably, intestine contains the highest concentration of IgA-producing cells, which secrete several grams of antibody into the lumen per day [193]. Moreover, sIgA could be produced in a T cell-independent manner against the commensal bacteria, principally by factors produced by DC, or IL-10, retinoic acid, and IgA-inducing protein, among others [175]. Transcytosis of dimeric IgA antibodies across epithelial cells is mediated by the polymeric Ig receptor [194]. In the mucosa, sIgA has different functions: it binds and retains bacteria in the mucus (immune exclusion), targets the bacterial surface and toxins (neutralization), and facilitates antigen uptake [195].

In summary, the intestinal barrier functions in a coordinated manner to regulate the intestinal immune response with the aim of preventing systemic infection and maintaining overall health. In this sense, it is important to know that diet significantly influences the interplay between microbiota and immune response, by providing substrates for intestinal bacteria and directly modulating immune cells. In turn, the barrier function acts as a critical line of defence against pathogens and allergens from the diet, facilitating nutrient absorption while preventing the entry of antigens and microorganisms into the body [185]. In addition, the internal immunological barrier allows peaceful coexistence with intestinal microorganisms, orchestrating appropriate inflammatory and defensive responses to threats [196]. Dysfunction of the intestinal barrier, whether due to increased epithelial permeability or an imbalance in the composition of the intestinal microbiota, known as dysbiosis, has been implicated in inflammatory disorders and food allergies, and contributes to the progression of diseases such as cancer, obesity, diabetes, or neurological disorders, for example [197].

### 3.2.2. Disruption of intestinal function

The integrity of the intestinal barrier and the health of the gut microbiota are crucial for maintaining overall health and preventing inflammatory disorders. Different insults such as diet, alcohol, drugs, toxins, stress, and pathogens can significantly influence the balance of the intestinal microbiome, intestinal permeability, and immune response [198,199]. The intestinal barrier functions as a sophisticated defence mechanism, with its components working together in a coordinated manner. The major glycolytic component of the outer membrane of Gram-negative bacteria from microbiota are bacterial lipopolysaccharides (LPS), which are recognized as endotoxins and can trigger inflammatory responses via TLRs [200]. When intestinal permeability is compromised, there is an increase in bacterial/LPS translocation to inner intestinal layers, leading to local inflammation and worsening of barrier dysfunction in a vicious cycle (figure 3). Reduced microbiota diversity can also result in a thinner mucus layer, promoting increased interaction between pathogens and the epithelium [180]. Consequently, the mucosal immune system must respond by the release of proinflammatory cytokines, as well as the recruitment of additional immune cells to the site of inflammation. Prolongated intestinal inflammatory response is associated with metabolic alterations such as obesity, metabolic syndrome, and insulin resistance. Moreover, several studies have reported that increased permeability of the intestinal epithelium can facilitate the entry of potential food allergens, thus heightening sensitization and allergy risks [201,202]. Additionally, systemic circulation of LPS due to inadequate neutralization by the intestinal immune system leads to metabolic endotoxemia and systemic chronic low-grade inflammation [203]. In this sense, microbiota dysbiosis, barrier dysfunction and low-grade inflammation are commonly observed in several disease states including cardiovascular disease (CVD), cancer, obesity, respiratory disease, diabetes, inflammatory bowel disease (IBD), brain disorders, chronic kidney disease and liver disease. Although their causal role in the development of disease has not been established, treatments have been proposed to improve disease status by restoring gut health [88].

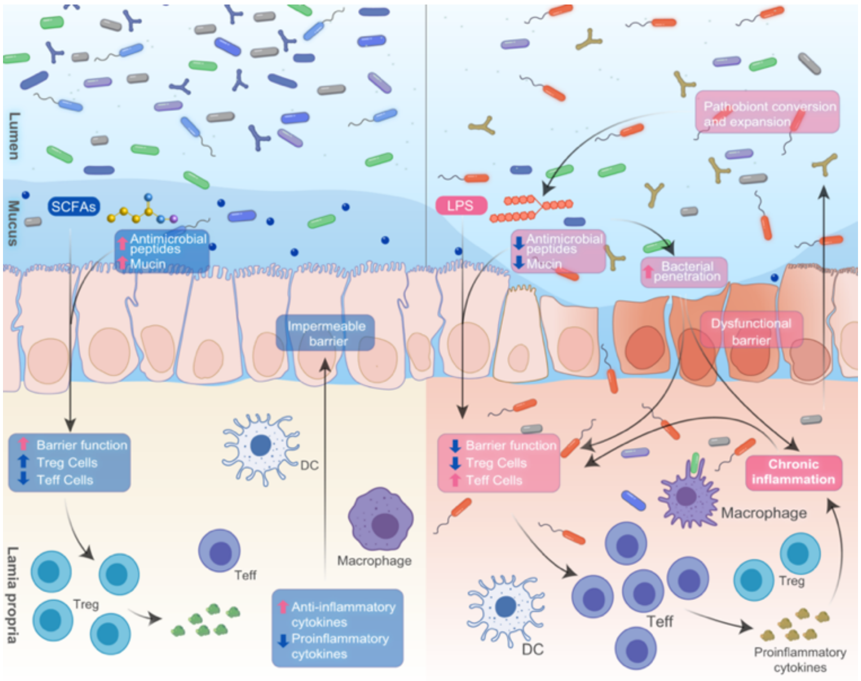


**Figure 3**. Intestinal barrier in healthy and disease state. In a healthy state, the barrier is maintained by a balance of microbiota, mucus layers and short-chain fatty acids (SCFAs), promoting a robust epithelial layer and activation of Treg cells that secrete anti-inflammatory cytokines. In disease, the disrupted barrier allows passage of LPS and bacteria, leading to activation of T effector cells (e.g. Th1, Th2, Th17), increased pro-inflammatory cytokines, leading to chronic inflammation and exacerbation of barrier dysfunction. Adapted from Hou *et al.*[204].

### Models of inflammation and disrupted barrier: Cafeteria diet and LPS injection

It has been widely described that a balanced diet is essential for maintaining a healthy gut microbiome, intestinal barrier integrity and immune tolerance. In contrast, an unbalanced diet, such as the Western diet, can lead to reduced microbiota diversity, dysbiosis, altered barrier function and chronic inflammation [150]. In the laboratory, the Cafeteria Diet (CAF) is used to mimic the human Western diet in animal experiments, by consisting of highly palatable and energy-dense foods like hot dogs and muffins [205]. Compared to other high-calorie diets, the CAF has become the preferred choice for studying diet-induced obesity and metabolic syndrome in animal models [206]. Rats fed a CAF for five weeks showed greater weight gain compared to controls, and some other changes began to increase [207].

This diet is characterised by high levels of simple carbohydrates and fats, which are associated with dysbiosis, and low levels of fibre, vitamins and minerals, nutrients that play a role in proper barrier function. In this sense, the intestinal permeability observed in obesity has been linked to microbial dysbiosis, dietary patterns (high sugar, high fat) and nutritional deficiencies [200]. On the one hand, reduced intake of dietary fibre leads to reduced microbial diversity [208], while high consumption of simple sugars and fats leads to a decrease in beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and an increase in LPS-rich Gram-negative bacteria [209]. On the other hand, these diets are associated with disrupted intestinal crypts, reduced goblet cell numbers and changes in mucin production. In addition, due to their low fibre content, the microbiota catabolizes endogenous proteins and mucus glycoproteins, leading to increased levels of harmful metabolites and mucus degradation [210]. Furthermore, at the level of the epithelial layer, chronic overconsumption of this type of diet promotes the loss of TJ proteins [211]. Finally, data from animal studies suggest that dietary fat is the main macronutrient responsible for postprandial endotoxemia and that both the quantity and quality of dietary fat have different effects on metabolic endotoxemia [212]. In this sense, the persistently high circulating levels of inflammatory cytokines associated with the obesity induced may also alter the structure and localisation of TJs [213].

This reduction in mucus and alteration in permeability leads to a translocation of luminal contents to the *lamina propria* and enhances the host immune response to the microbiome by allowing unrestricted bacterial access to the epithelium [12], resulting in an increase in bacterial endotoxins in the systemic circulation and intestinal and general inflammation [150]. The disruption of the intestinal homeostasis affects Tregs cells, resulting in the loss of immune tolerance and aberrant effector responses, with increased secretion of pro-inflammatory cytokines via Th1 and Th17 pathways [214]. Consequently, blood monocytes are recruited to inflamed tissues where they release a variety of proinflammatory mediators, such as TNF-α, macrophage infiltrating factor, IL-1β, IL-6, IL-12, and IL-18 [156]. In addition, persistent neutrophil activation, increased infiltration and low apoptosis rates are common in this situation, contributing to the chronification of intestinal and systemic inflammation [158].

Thus, the Western or cafeteria diet causes intestinal damage with subsequent systemic inflammation due to the entry of microbiota-derived endotoxins into the bloodstream, leading to diseases such as obesity or metabolic syndrome [215]. This situation is also addressed in the laboratory with LPS or dextran sodium sulphate (DSS) treatments in animal models, *ex vivo* human intestinal tissues and cell culture studies [216–219]. Intraperitoneal injection of LPS induces a systematic and local inflammatory response that affects intestinal permeability and exacerbates inflammation [218,219]. Chronic low-grade inflammation associated with metabolic endotoxemia is an important factor in the development of many chronic diseases in animals and humans [212,220].

### Intestinal barrier and food allergy

Intestinal immune barrier function is crucial in defending against allergens, and disruptions in this barrier can contribute to the development or exacerbation of food allergies.

Normally, tolerance to food antigens develops early in life, supported by factors such as breast-feeding, which transfers immune mediators from mother to child. This process involves the participation of DCs and differentiation of naïve T cells into Treg cells, as well as the production of IL-10, and sIgA or sIgG, that inhibit sensitization to specific food allergens and promote immune tolerance [221]. However, the disruption of physiological tolerance leads to the production of proinflammatory cytokine like IL-4, IL-5, and IL-13 by intestinal epithelial cells and DCs, shifting the differentiation of naïve lymphocytes toward allergic responses (through Th2) rather than tolerance [72].

This allergic response consists in the expansion of mast cells, basophils, and, consequently, eosinophils in the mucosa, accompanied by the switching to IgE-producing B cells. Mast cells, key cells in allergy development, express on their surface the Fc$\varepsilon$RI, a high-affinity IgE receptor, to which the produced allergen-specific IgE binds during the sensitization phase. Subsequent exposure to the same food allergen cross-links allergen specific IgE on mast cells, triggering an effector phase response (degranulation) characterized by the release of chemical mediators, such as histamine, prostaglandins, and leukotrienes, which induce allergic symptoms [222,223]. Therefore, increased total IgE production is strongly associated with allergic response, making plasma IgE levels a reliable marker when IgE-mediated food allergy is suspected [224].

Research into disease origins highlights various factors predisposing, initiating, or exacerbating altered immunity in allergic diseases, including inherent epithelial barrier dysfunction, loss of immune tolerance, disturbances in the gut, and organ-specific microbiomes, diet, and age [202]. Thus, gut barrier function plays a pivotal role in either preventing or facilitating food allergy development. An altered barrier, increased permeability, dysbiosis, or dysregulation of the mucosal immune system can contribute to the development or exacerbation of food allergies [225]. Firstly, a leaky gut can allow higher levels of allergens to cross the epithelium and reach immune cells, particularly mast cells, stimulating allergic reactions [226]. Secondly, a healthy microbiota promotes immune tolerance to harmless food proteins by promoting Treg cells, but microbiota imbalance can overactivate the immune response to those antigens [227]. Additionally, dysregulation of immune response can result in aberrant reactions to dietary proteins. Furthermore, this epithelial barrier disfunction and the chronicity of inflammatory state, could lower the threshold for sensitization to innocuous substances because of the inflammatory environment, potentially precipitating allergic sensitization [228]. Once again, there is also evidence that mediators released from mast cells and other Th2 cytokines contribute to the increase of paracellular permeability, being a vicious cycle [229].

Intestinal inflammatory-related diseases are characterized by an increase of mast cells and their degranulation products in the small intestine and colon [230]. LPS treatments also enhance the Th2 response with an increase in IgE secretion, mast cell recruitment and degranulation, and eosinophil activation [218,231].

In this sense, dysregulation or disturbance of the intestinal barrier could produce bowel inflammation, as well as exacerbate other inflammatory-related pathologies at systemic level.

### 3.2.3. Dietary interaction at the intestinal barrier

As mentioned above, diet, long recognised as one of the most important external factors influencing health, has a primary impact on intestinal health. Dietary components interact directly with the gastrointestinal tract and therefore influence its integrity and composition [232]. While the typical Western diet has been shown to result in a permeable intestine and chronic inflammation; a nutritionally balanced diet, rich in fruits and vegetables, is essential for maintaining a healthy gut microbiome, preserving the integrity of the intestinal barrier, immune tolerance, and maintaining normal gut physiology. In addition, consumption of fruits and vegetables in general, but also plant proteins and peptides, polyphenols, $\omega$-3 fatty acids, and dietary fibre have been associated with intestinal health benefits [233]. Consequently, our dietary choices influence the structure, composition, and function of the microbiota, which interact with the intestinal epithelium and mucosal immune system and to maintain intestinal homeostasis in a healthy state [150].

In the context of dysbiosis, some microbiota-based therapies, including prebiotics and probiotics, have emerged as promising treatments [234]. Probiotics, such as *Lactobacillus* and *Bifidobacterium*, are live microorganisms that have one or more beneficial effects on the host when consumed in sufficient quantities, whereas prebiotics are compounds that are indigestible by the human GIT and are broken down by microbiome or probiotic microorganisms [235]. Major sources of prebiotics include fruits, vegetables, legumes, and whole grains, which contain dietary fibre and other fermentable carbohydrates, also known as microbiota-accessible carbohydrates (MACs) [233]. It has been described that the availability of insoluble fibre and MACs could influence the composition of the microbiota. Furthermore, their consumption has been associated with improved paracellular permeability by upregulating TJs protein expression [236]. Finally, MACs consumption has been shown to ameliorate low-grade inflammatory situations induced by a high-fat diet [183]. On the other hand, an insoluble dietary fibre such as insect chitin has been widely linked to present health benefits, by promoting the growth of beneficial bacteria and inhibiting the growth of some potentially pathogenic bacteria. Additionally, chitin derivatives have also been shown to have anti-inflammatory properties [237].

Several studies have shown that bioactive peptides derived from dietary proteins can influence gut health by modulating the barrier function. In this sense, they have been described to stimulate the mucus production and secretion, increase IgA secretion, and induce epithelial cell proliferation [238]. Moreover, peptides can affect from different protein sources have been related to increasing the microbiota diversity and regulating the immune response [239]. Other authors also note that glutamine consumption could improve intestinal health by participating in epithelial cell renewal and upregulating the expression of TJs [240]. However, more research is needed into the efficacy of bioactive peptides and amino acids from diverse and novel protein sources, such as insects, in the treatment or prevention of disease.

Other bioactive compounds such as polyphenols, mainly found in plant sources, exert various beneficial effects on gut health and immune function. They have been suggested to improve intestinal integrity by modulating the organization of TJs and increasing their expression, ameliorating intestinal barrier defects and metabolic endotoxemia induced by a high-fat diet [241,242]. Similarly, $\omega$-3 fatty acids have been described to have these properties, and also to be involved in the regulation of signalling pathways to reduce epithelial inflammation, by attenuating the production of pro-inflammatory mediators and upregulating the production of pro-resolving mediators [243].

In conclusion, diet is a key factor influencing intestinal health, with certain foods and nutrients known to enhance intestinal barrier function [236]. Further research into these dietary components, as well as foods that may contain them, could provide valuable insights into promoting gut health and preventing gut disease. Furthermore, with all the evidence from the previous sections, we could conclude that the intestinal barrier is a dynamic interface that regulates the entry of food and pathogens into the GI tract, affecting overall health maintenance.

Finally, in terms of food recognition, the dietary components that reach the intestine interact with taste receptors, that are not limited to the oral cavity but are also present in the intestinal tract [6]. As described in the following section, they have been implicated in signalling pathways associated with physiological functions beyond their role in taste and have been suggested to influence various metabolic and immune responses [244]. However, the effect of different dietary patterns, such as new protein sources, on the intestinal taste receptors and the consequent effects on the intestinal barrier, but also on overall health, needs to be further investigated and will be addressed in the present thesis.

### 3.3. Taste Receptors

The consumption of food components activates a cascade of responses within our body, intricately influencing our body homeostasis. Traditionally, the sense of food compounds has been carried out by taste receptors, specialized proteins predominantly located in taste buds dispersed throughout the oral cavity [245]. These receptors play a pivotal role in recognizing exogenous compounds present in food and beverages, as well as other ingested substances. The signals they generate are transmitted via afferent gustatory nerves to brain structures responsible for central taste processing, shaping our sensory experience of flavour [246]. However, the influence of taste receptors extends far beyond the confines of the oral cavity. Emerging research has revealed their presence in extra-oral tissues and organs, spanning from the brain and skin to the reproductive system and gastrointestinal tract [247]. The abundance and tissue distribution of taste receptors raises the prospect of their involvement in other physiological functions [248].

Taste receptors are sensitive to different tastes, including sweet, bitter, salty, sour and umami. The salty and sour tastes are transduced by ion channels, while the epithelial sodium channel (ENaC) responds to sodium stimuli, the proton-selective ion channel Otop1 and the decrease in pH are candidates for sour transduction signals [249,250]. In contrast, umami, sweet and bitter receptors belong to the G-protein-coupled receptor (GPCR) superfamily and are divided into two types. Standard names follow the description of “taste receptor, type X, member X”, abbreviated in capital letters/ upper case (TASXRX) for humans, and lower case for rodents (TasXrX) [251,252]. TAS1Rs, responsible for sweet and umami taste, are GPCRs with a long amino-terminal and function in dimers using TAS1R3 as the obligate subunit. Specifically, the TAS1R2-TAS1R3 heterodimer is responsible for sensing sweetness, while L-amino acids and ribonucleotides interact with TAS1R1-TAS1R3 to form the taste sensation known as umami [253,254]. Bitter compounds are sensed by TAS2R, also GPCRs with short amino termini, and can act as monomers [255] . In the following paragraphs, TAS1R and TAS2R will be described in more detail, as they have been one of the subjects of study in this thesis.

The taste pathways through taste receptors belonging to the GPCRs family, that is to say sweet, umami and bitter transduction mechanism, begin with the binding of the taste ligand to the specific receptor complex, inducing a conformational change (figure 4). Then, the GTP-bound heterotrimeric G proteins (α-, β-, and γ-subunits) dissociate and β- and γ- subunits activate the membrane-bound phospholipase C2 (PLCβ2), thus inducing the production of inositol-1,4,5-triphosphate (IP3) and diacylglyceride (DAG) [248,256,257]. IP3 binds to its receptor located in the membrane of the endoplasmic reticulum, thus activating calcium channels, and leading to an increase in cytoplasmatic Ca$^{2+}$ ions. This calcium activates transient receptor potential cation channel subfamily M member 5 (TRPM5) [258] which, together with other Na$^{+}$ channels, increases the Na$^{+}$ ions. Cells respond to the sodium signal via depolarisation and extracellular efflux of the neurotransmitter, adenosine-5'-triphosphate (ATP), to afferent gustatory nerves through the calcium homeostasis modulator 1 CALHM1 or CALHM1/CALHM3 and leading to taste perception [259]. However, it is suspected that this is not the only way the signalling pathway is activated, due to α-gustducin subunit dissociation when a ligand binds to GCPR seemed to also elevate the intracellular levels of Ca$^{2+}$ via hydrolysis of cAMP, which again results in the exocytosis of neurotransmitters [260,261]. In other locations for example, in the intestine, these signalling pathways have been described to finish with the release of hormones or other mediators of paracrine function [262].

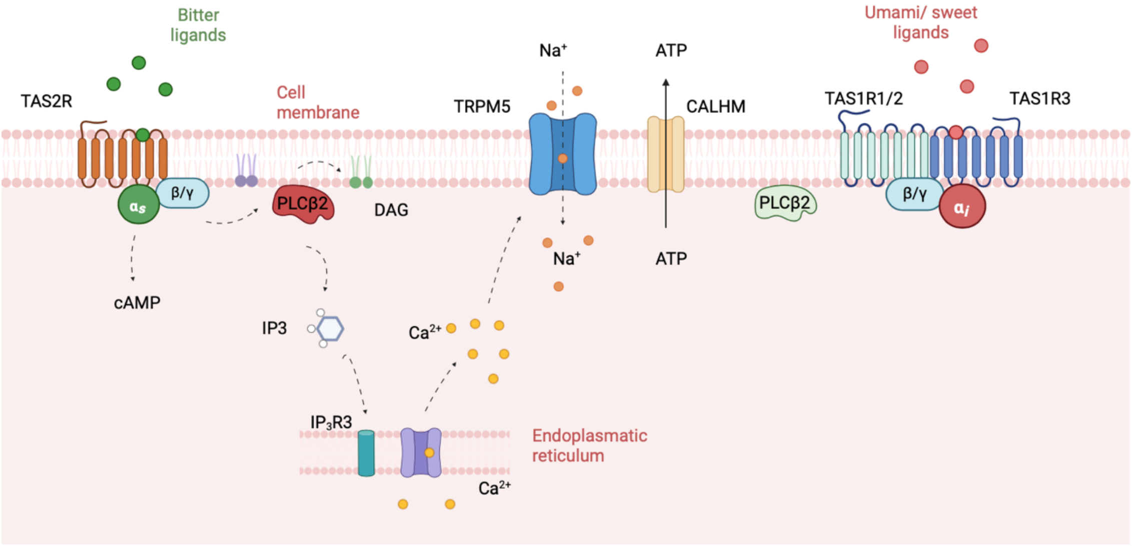


**Figure 4.** GPCR taste receptor signalling pathway: when the ligand binds TAS1R1/TAS1R3, TAS1R2/TAS1R3 or TAS2R, G proteins dissociate to activate the production of IP3, the secretion of Ca$^{2+}$ from the endoplasmic reticulum and the consequence gradient of Na$^{+}$. This results in the secretion of ATP and among other effector molecules, that induce taste perception or lead to the activation of different functions. Created with BioRender.com.

### Sweet and umami taste receptors (TAS1R for humans, Tas1r for rats)

TAS1R, which comprises taste receptor type I member 1 (TAS1R1), 2 (TAS1R2) and 3 (TAS1R3), function as heterodimers, each possessing a large extracellular domain fused to the heptahelical seven transmembrane domain [263].

TAS1R2/TAS1R3 interacts with sweet-tasting compounds such as sucrose, fructose, artificial sweeteners, and certain D-amino acids, eliciting the sweet taste sensation [263]. Extra-oral localisation of this receptor has been described to confer it involvement in various physiological processes; for instance, it plays a role in insulin secretion, tissue growth, and regeneration of skeletal muscles, thereby contributing to the healthy processing of sweet compounds [264]. Their participation in insulin secretion may occur directly, due to its expression in pancreatic $\beta$-cells, or through their involvement in GLP-1 release, because of their expression in enteroendocrine L-cells [265,266].

On the other hand, the term "umami" originates from the Japanese word "umai" meaning "delicious" [246], and is associated with foods such as beef, cured meats, mushrooms, soy sauce, aged cheese, seafood, and tomato [267]. Thus, TAS1R1/TAS1R3 agonist included L-amino acids, such as glutamate, aspartate, alanine, serine, asparagine, arginine, histidine, threonine, glutamine, and 5′-ribonucleotides, (inosine 5′ monophosphate; IMP), and also some peptides, with L-glutamate being the principal stimulus for umami taste, often found in the diet as monosodium glutamate (MSG) and its salt form [263].

Extra-oral umami receptors have been linked to the modulation of hormonal release (i.e., ghrelin and cholecystokinin), due to their stomach and intestinal location [268]. Moreover, some evidence suggests their involvement in inflammatory and intestinal function regulation [253,269].

### Bitter taste receptors (TAS2R for humans, Tas2r for rats)

The bitter taste sensation can be triggered by wide array of compounds with diverse chemical structures, from simple salts to large complex molecules, many of which are considered toxic [255]. Humans express approximately 26 bitter taste receptor subtypes, while rats express around 37 different subtypes [251,270], each with its own unique nomenclature as previously reported by Descamps-Solà *et al.* [6]. Furthermore, each subtype is susceptible to activation by specific bitter compounds or a broad spectrum of them [271]. In this sense, a large list of agonists specific for some bitter receptors have been identified, including food compounds such as resveratrol, epicatechin gallate, quinine, and artificial sweeteners such as acesulfame K and saccharin [272,273]. Additionally, certain TAS2R have demonstrated sensitivity to specific peptides and amino acids [274–278].

Beyond their role in taste perception, their presence in hearth, bladder, airway, and gastrointestinal smooth muscle supported their participation in muscle relaxation and bronchodilatation [268]. Additionally, TAS2R is also located in epithelial intestinal cells and have been demonstrated their participation in food intake decrease through changes in the hormonal secretion [264,279]. Furthermore, these receptors have been also associated to intestinal defence mechanisms and immune response [280].

### 3.3.1. Intestinal Taste Receptors

Bitter, sweet and umami taste receptors have been reported to expressed in the intestine and have been found to detect a wide array of endogenous molecules and dietary nutrients within the gut lumen. In this sense, the composition of the intestinal luminal content varies considerably with diet, necessitating that the intestinal epithelium senses and responds to these significant changes to regulate its functions accordingly [281]. These taste receptors are considered to be activated by certain nutrients and play important roles in various intestinal physiological activities, through similar chemosensory networks as lingual receptors [262].

Thus, principally in the small intestine, they are primarily located on the enteroendocrine cells. Upon activation by specific ligands, both type 1 and type 2 taste receptors have been shown to trigger the release of various hormones and neurotransmitters, such as glucagon-like peptide 1 (GLP-1) [282], cholecystokinin (CCK) [283], and ghrelin [284,285]. These molecules modulate nutrient absorption and metabolism, satiety, food intake, eating behaviour, and intestinal motility, thereby maintaining homeostasis in response to dietary components [286–288]. This evidence reinforces the importance of intestinal function in general health beyond the intestine. For example, malfunction of intestinal taste receptors may lead to digestive disorders, and result in obesity, type 2 diabetes, as well as gastrointestinal diseases [245,253,289]. Moreover, genetic alteration in TAS2R38, have been linked to intestinal dysbiosis, altered diet uptake and impaired regulation of innate immunity, that could lead to other alterations such as Parkinson's disease [290].

On the other hand, TAS1R and TAS2R have also been found on several other cell types in the intestinal epithelium, such as enterocytes, Tuft, Paneth, goblet and microfold cells, thus, intestinal taste receptors are involved in the regulation of gut barrier function and immune responses [256,291]. Intestinal Tuft cells have recently been associated with participating in the initiation of type 2 immunity through the release of IL-25, after activation of intestinal bitter taste receptor by parasites or microbes [292]. Furthermore, Howitt *et al.* determined that Tas1r3 has important roles in regulating Tuft cell homeostasis in the small intestine, thereby modulating sensitivity to luminal stimuli during the expansion of type 2 immune response [293]. On the other hand, their presence in the Paneth and goblet cells suggested their participation in the antimicrobial peptides and mucus production [280,294].

Some studies have been linked the alteration of TASR or its downstream signalling molecules, with intestinal impairment and systemic inflammatory response. For example, DSS-induced colitis in α-gustducin knockout mice had aggravated IBD sympthoms, augmented immune cell infiltration and inflammatory mediators release than the wild-type control mice [295]. Conversely, another study on the activation of TAS2Rs and metabolic response, observed a reduction in proinflammatory cytokines that also suggested the regulation of anti-inflammatory responses through these receptors [287]. Moreover, TAS2Rs expression in macrophages, monocytes, mast cells, neutrophils, and in B and T cells, leads us to think about their role in innate and adaptative immunity [256]. Therefore, activation of these receptors modulates epithelial immune function, mucus secretion, and antimicrobial peptide production, influencing the composition of the gut microbiota and immune homeostasis, locally and beyond the gastrointestinal tract [296].

### 3.3.2. Taste Receptors and Immune Function

In addition to the gastrointestinal tract, taste receptors have been extensively implicated in the immune response in other locations. TAS2R have recently been found in peripheral blood neutrophils, monocytes, and also in resting and activated lymphocytes, and have been described as contributing to adaptative response [297]. Likewise, human lung macrophages have been reported to express bitter taste receptors and treatment with bitter agonists supressed cytokine production [298]. Both TAS1R and TAS2R have been found in circulating leukocytes [299]. TAS2R have been implicated in anti-inflammatory modulation through the regulation of IL-6 release, in human gingival fibroblasts [273]. Similarly, gingival TAS2R respond to bacterial signals that induce host innate immune responses, such as secretion of AMPs, to prevent oral bacteria overgrowth and regulate oral microbial composition [300]. Hollenhorst *et al.* suggested that bitter taste sensing in tracheal brush cells triggers the innate immune response to bacterial infection [301]. Furthermore, a study in urethral brush cells showed that activation of bitter and umami receptors leads to the release of acetylcholine and consequently to the elimination of pathogens from the urinary tract [302].

On the other hand, acute inflammation but also a chronic low-grade inflammation associated with pathologies such as obesity, appeared to alter the taste system. Moreover, the immune role of taste receptors highlights further links between immunity and taste [303]. In obesity, there is considerable evidence to suggests that taste perception is altered and that TAS1R, TAS2R, or their associated transduction signalling molecules are involved in regulating inflammatory status, and may also act as a negative feedback signal to alter nutrient intake to maintain energy homeostasis [304].

### 3.3.3. Modulation of Taste Receptors Gene and Protein Expression

Besides activation or inhibition, it has been reported that the expression of intestinal taste receptors can be modulated by diet and changes in pathological situations, with obvious consequences for their physiological response [305]. At the oral level, chronic sucrose exposure inhibits taste responses to higher concentrations of sweet stimuli, suggesting that a receptor may be sensitised and downregulated after chronic consumption of an agonist [306]. Similarly, long-term overexposure to appetitive tastes, such as monosodium glutamate or saccharin, leads to a downregulation of the specific receptors (TAS1R1 or TAS1R2, respectively) in the taste buds of animals, raising the possibility that targeting taste receptor modulation may offer potential strategies to combat obesity [307]. On the other hand, Lin *et al.* showed an increase in Tas2r gene expression in the tongues of inflammation-induced mice, resulting in an altered bitter taste and the possibility of more infections [308]. However, this modulation of receptor expression has also been observed in extra-oral tissues, and some authors have investigated modulation at the gut level. For instance, Zhang *et al.* reported increased intestinal mRNA expression of Tas1r2 and Tas1r3 when rats consumed sucrose and fructose with or without high-fat diet [309]. Likewise, Wang *et al.* described that obese humans and animal models have altered intestinal expression of TAS1R and TAS2R, and consequently altered chemosensory signalling, such as ghrelin secretion [310]. In addition, in a mouse model of type 2 diabetes induced by a high fat diet and streptozotocin, colonic Tas1r3 and TRPM5 expression was increased, whereas a quinoa diet as a corrective treatment reversed these changes [311].

As previously been described, the microbiota has a major impact on intestinal function. Thus, a relatively new concept has been proposed that the gut microbiota has a role in taste receptor-mediated inflammation. Changes in the microbiota could affect the expression of taste GPCRs and their downstream signalling, thereby altering the innate immunity and host metabolism [304]. Swartz *et al.* reported an upregulation of intestinal TAS1R3 gene and protein expression in germ-free mice, associated with an increased preference for sucrose solutions [312]. Moreover, SCFAs derived from microbiota fermentation, have been described to influence TAS1R expression [313]. Similarly, another recent study has demonstrated a microbiota-dependent upregulation of Tas2r in mice subjected to a long-term high-fat diet [314], while Latorre *et al.* observed that antibiotic treatment reversed diet-induced T2R138 upregulation [305]. Because it is well known that diets alter the microbiota composition [315], it is reasonable to suspect that this could influence the expression of taste receptors.

All this research suggests that the expression of taste receptors in the gut may be affected by specific dietary compounds and pathologies, but the evidence remains limited. Further studies are needed to explore how different diets or dietary compounds modulate taste receptor expression and potentially affect physiological function. Given the importance of a healthy gut in preventing chronic inflammation, metabolic dysfunction and inflammatory diseases, and the intriguing link between intestinal taste receptor function, intestinal barrier integrity and systemic health outcomes, research in this area is critical. Understanding the relationship between dietary components, gut function, taste receptor expression and systemic responses is essential to develop targeted dietary strategies that promote overall health and well-being.

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# INTRODUCCIÓ

## 1. Preàmbul

La recerca de fonts proteiques sostenibles que no només contribueixin a reduir l'impacte ambiental de la producció d'aliments, sinó que també puguin beneficiar la nostra salut, és un objectiu global. En aquest context, els insectes s'han postulat com una font proteica d'alta qualitat, ja que aporten altres nutrients beneficiosos a part de les proteïnes, així com perquè la seva producció es considera respectuosa amb el medi ambient [1]. Així doncs, suposen una bona alternativa per a les fonts convencionals de proteïnes, però hi ha algunes barreres com la seva acceptació social. En aquest sentit, altres estudis que demostrin els efectes del consum d'insectes sobre la salut, i que permetin elucidar-ne els beneficis, són crucials per augmentar la seva acceptació [2].

Els components dietètics són un dels factors més importants que influeixen en la salut. En aquest sentit, la barrera intestinal serveix com a primera línia de defensa contra antígens, patògens i toxines luminals, alhora que facilita l'absorció de nutrients i la vigilància immunitària [3]. La disrupció de la integritat de la barrera intestinal, ja sigui per factors dietètics, disbiosi o desregulació immunitària, pot tenir conseqüències per a la salut sistèmica. Per exemple, en l'obesitat, el consum excessiu de dietes altes en greixos i sucres pot alterar la integritat de la barrera intestinal i promoure la inflamació de baix grau, contribuint a la disfunció intestinal i l'endotoxèmia metabòlica sistèmica 4. No obstant això, se sap que una dieta equilibrada rica en fibra i compostos bioactius afavoreix i manté una funció intestinal saludable [5].

Pel que fa al reconeixement dels aliments, els receptors del gust s'han identificat com a dianes potencials dels components dels aliments. Aquests receptors es troben en diversos teixits extra-orals, inclòs l'intestí, i s'associen amb la detecció de continguts luminals i respostes epitelials moduladores, que s'ha descrit que regulen la funció entero-endocrina i immune [6]. A més, diferents estímuls com els components dels aliments, la patologia o l'edat, podrien modular l'expressió dels receptors del gust i influir en les seves funcions fisiològiques [7].

A la llum d'aquesta evidència, explorar la relació entre la dieta, la salut intestinal i el benestar general, s’obre com una bona via d’investigació. Per tant, l'objectiu d'aquesta introducció és revisar els coneixements actuals sobre (1) la sostenibilitat de la producció d'insectes, els beneficis i preocupacions del consum d'insectes i el seu possible impacte en el manteniment de la funció intestinal; (2) l'estructura del tracte gastrointestinal, la funció de la barrera intestinal i la seva modulació; així com (3) la funció dels receptors del gust, les implicacions intestinals i immunitàries de la seva activació, i la seva possible modulació per la dieta.

## 2. Els insectes com a font alternativa de proteïnes

### 2.1. La necessitat de trobar fonts alternatives de proteïnes

La població mundial s'està expandint a causa d'una combinació de factors, incloent la disminució de les taxes de mortalitat a nivell mundial, les elevades taxes de fecunditat en regions específiques i el fenomen de l'impuls demogràfic. En conseqüència, les projeccions indiquen un augment de 1,3 milions de persones en els propers 30 anys, que culminarà en un valor estimat de 9,7 milions per al 2050 [8]. Per tant, la producció d'aliments ha d'augmentar, i una preocupació fonamental en aquest context és l'escalada de la demanda de proteïnes, que es preveu que hagi augmentat un 70% aproximadament entre el 2012 i el 2050 [1].

Entre l'espectre de macro i micronutrients essencials derivats de la dieta, la proteïna assumeix un paper crític en la formació de teixits, els processos de reparació i la constitució dels enzims i hormones [9,10]. L'aportació adequada de proteïnes representa el 10% de l'aportació energètica i la seva qualitat està íntimament lligada a la presència d'aminoàcids essencials, sent les fonts d'origen animal tradicionalment reconegudes per la seva qualitat superior en comparació amb la majoria de les fonts vegetals [11]. Ara bé, patrons dietètics occidentals, caracteritzats per un consum elevat de productes lactis, carn i ous; sovint superen els llindars dietètics recomanats per als greixos saturats i la carn vermella. L'adherència prolongada a aquestes dietes es correlaciona significativament amb l'augment de les taxes de mortalitat [12]. No obstant això, els ous, els làctics, la carn, el peix i els seus derivats, quan es consumeixen en quantitats adequades, destaquen com a fonts convencionals de proteïnes que també contenen altres nutrients essencials com lípids, vitamines i ferro [11]. Això els posiciona com a opcions altament nutritives, especialment en regions on l'accés als aliments és limitat, i protegeix contra la desnutrició [13]. Així doncs, la seva producció ha experimentat un creixement exponencial per contrarestar la gran demanda de proteïna d'alta qualitat, i continuarà augmentant, principalment la producció ramadera.

Avui en dia, és ben sabut que la ramaderia contribueix al voltant del 15% de les emissions totals de gasos d'efecte hivernacle (GEH) [14], principalment de la producció bovina i ovina, tant directa (a través de la producció de metà pels remugants) com indirecta (a través de les emissions reactives de nitrogen i diòxid de carboni, i la desforestació associada a la producció de pastures i cultius farratgers) [15]. A més, la contaminació i l'escassetat d'aigua també estan vinculades a aquesta producció d'aliments per a animals, posicionant-la com un dels principals contribuents al canvi climàtic [16].

Per tant, el gran repte és alimentar una població humana en expansió enfocant la producció en aliments respectuosos amb el medi ambient [14,17]. En aquest sentit, els mètodes convencionals de producció d'aliments s'estan re avaluant davant d'aquest repte, donant lloc a un interès creixent per l'agroecologia. Aquest enfocament té com a objectiu transformar els sistemes productius actuals per potenciar la seva sostenibilitat i reduir la seva petjada ambiental [18]. Un altre aspecte d'aquesta transició passa per reduir el consum de carn i productes lactis. Nombrosos estudis han emfatitzat la importància d'aquest canvi dietètic en la consecució dels objectius de canvi climàtic [19]. Com s'ha esmentat abans, el sector ramader contribueix significativament a les emissions de gasos d'efecte hivernacle, i la seva reducció podria tenir un impacte substancial en la mitigació del canvi climàtic. No obstant això, en la recerca de l'adequació nutricional global, hi ha un interès creixent en explorar mètodes sostenibles per produir fonts de proteïnes noves i d'alta qualitat, que puguin servir tant com a aliment humà com ser ingredients per a l'alimentació animal. Això inclou explorar fonts de plantes actualment infrautilitzades, insectes i organismes unicel·lulars, com bacteris, algues i fongs [16], així com *"carn" cultivada in vitro* 14. Aquestes alternatives ofereixen la possibilitat de diversificar o substituir parcialment les fonts càrnies tradicionals, en línia amb els objectius de sostenibilitat i reducció de l'impacte ambiental.

## 2.2. L'impacte ambiental de les diferents fonts de proteïna

Algunes revisions interessants s'han centrat en descriure els beneficis ambientals de les fonts de proteïna que representen alternatives a la carn [21–24]. Les carns remugants, per exemple, tenen impactes que són de 3 a 10 vegades més elevades que d'altres aliments d'origen animal i de 20 a 100 vegades que els d'origen vegetal, per a tots els indicadors examinats [25].

En aquest context, les fonts vegetals semblen ser un substitut acceptat, ja que estan presents de manera natural en la dieta de les persones, aporten beneficis per a la salut i el medi ambient i tenen menors costos de producció associats. Com a font de proteïna vegetal s'inclouen plantes lleguminoses (per exemple, soja, llenties, lupins, cigrons), plantes oleaginoses, cereals (blat de moro, arròs, blat) i pseudocereals (amarant i quinoa) [24,26]. Els estudis van demostrar que la producció d'1kg de carn de boví requereix 50 vegades més terra i unes 15 vegades més aigua que la producció d'1kg de gra i verdures. A més, s'ha descrit que la substitució del 25-50% dels aliments d'origen animal per d'altres d'origen vegetal resultaria en una reducció del 40% de les emissions de nitrogen i del 25-40% en altres emissions de GEH a la Unió Europea [12]. Tot i que la font vegetal també té un efecte beneficiós sobre la salut humana, reduint el risc d'algunes malalties cròniques, generalment són deficients en aminoàcids essencials [27]. Per tant, combinar fonts vegetals en l'equilibri adequat és una bona solució per aconseguir perfils d'aminoàcids essencials adequats. D'altra banda, la proteïna microbiana, o proteïna "única" cel·lular (SCP), s'estableix com una alternativa potencial ja que el cultiu de microorganismes no requereix un gran nombre de terres com en els cultius i la ramaderia, ja que els microorganismes solen conrear-se en tancs o reactors. La SCP inclou la proteïna derivada de microorganismes unicel·lulars o fins i tot pluricel·lulars, principalment fongs (llevats i fongs filamentosos), microalgues (cianobacteris i eucariotes unicel·lulars) i bacteris [22].

Finalment, encara que els insectes es troben entre les alternatives més controvertides a causa dels conflictes amb els hàbits culturals de diverses poblacions, estan presents en la dieta d'altres poblacions de tot el món i tenen nombrosos beneficis ambientals i nutricionals. Els estudis indiquen que els indicadors ambientals com l'ús del sòl, la petjada hídrica i les emissions de GEH són un 40-60% més baixos per a l'alimentació i l'aliment dels insectes comestibles en comparació amb la ramaderia tradicional [1,28,29]. A més, la capacitat dels insectes per alimentar-se de bioresidus, com ara residus de bolets o pells de fruites, redueix encara més l'impacte ambiental en convertir els residus en compost, la qual cosa també proporciona noves estratègies per a una bioeconomia circular [30,31]. Així mateix, l'eficiència de conversió de pinso, és a dir, l'aliment necessari per produir 1kg d'augment de pes corporal, difereix significativament entre el bestiar convencional i els insectes. Per exemple, els grills són més eficients a l'hora de convertir l'aliment en massa corporal en comparació amb la ramaderia tradicional. La relació de conversió d'aliment del pes comestible mostra que els grills són dues vegades més eficients que els pollastres, quatre vegades més que els porcs i dotze vegades més que el bestiar boví [32], cosa que suggereix que els insectes són una font alternativa amb més rendibilitat. Aquesta eficiència subratlla el paper dels insectes com a sistema alimentari sostenible, la qual cosa, a més del seu valor nutritiu, contribueix positivament als impactes ecològics, ambientals i econòmics [33].

Per tant, en termes d'impacte ambiental, col·lectivament, aquestes fonts alternatives de proteïna ofereixen una solució més sostenible que la ramaderia tradicional. Redueixen la pressió sobre la terra i els recursos hídrics i contribueixen a reduir les emissions de gasos d'efecte hivernacle. Aquest canvi cap a proteïnes alternatives és essencial per mitigar els impactes ambientals de la producció d'aliments i donar suport a la seguretat alimentària mundial. En particular, l'alta eficiència de conversió d'aliments, cicles de vida curts, les altes taxes de reproducció [34], i la capacitat de prosperar gràcies a una àmplia gamma d'aliments, inclosos els subproductes del processament d'aliments i els fluxos de residus d'alt impacte [35], fan dels insectes una de les opcions més beneficioses per al medi ambient i econòmicament viables [36].

### 2.3.Consum d'insectes per part dels humans, entomofàgia

Els insectes s'han consumit des de principis de l'evolució humana i més de 2000 espècies diferents d'insectes són consumides de manera regular per dos mil milions de persones a tot el món, principalment a Àfrica, Àsia, Oceania i Amèrica del Sud [26,37,38]. Aquesta pràctica de menjar insectes es coneix com entomofàgia [39], i inclou el consum d'escarabats (Coleoptera), erugues (larves de papallones i arnes; Lepidòpters), abelles, vespes i formigues (himenòpters), i llagostes, saltamartins i grills (ortòpters) [32]. Durant mil·lennis, els insectes han estat una font important de proteïnes dietètiques en algunes poblacions humanes [40]. No obstant això, als països occidentals, l'entomofàgia és poc freqüent i s'associa a una pràctica repugnant [41,42]. A causa d'aquesta barrera cultural, l'ús d'insectes processats com a ingredient en pols, que després s'incorpori a productes com hamburgueses, pasta o aperitius, podria millorar l'acceptació dels consumidors [43]. D'altra banda, més investigacions que verifiquin els beneficis a nivell nutricional de consumir d'insectes i els avantatges ambientals, també podrien millorar-ne el coneixement i l'acceptació per introduir-los a les nostres dietes [42]. Així, la proteïna dels insectes podria passar a formar part de la proteïna total consumida pels humans en un futur proper [44].

Aquesta tendència emergent en les societats occidentals va començar el 2013, quan l'Organització de les Nacions Unides per a l'Alimentació i l'Agricultura (FAO) va assenyalar la necessitat d'enfocar les pràctiques modernes de ciències dels aliments a augmentar el comerç, el consum i l'acceptació dels insectes [32]. El Reglament 2015/2283 del Parlament Europeu i del Consell de la Unió Europea, va marcar un moment crucial, incorporant insectes sencers i les seves parts en la categoria de nous aliments [45]. Addicionalment, el 2015, l'Autoritat Europea de Seguretat Alimentària (EFSA) va emetre una opinió científica sobre el consum d'insectes, proposant una llista d'espècies d'insectes amb alt potencial d'ús tant com a alimentació animal com a alimentació humana [46]. Actualment, l'EFSA ha expressat una opinió favorable sobre la seguretat del cuc groc de la farina (*larves de Tenebrio molitor*) [47], la llagosta migratòria (*Locusta migratoria*) [48], el cuc petit de la farina (*larves d'Alphitobius diaperinus*) [49] i el grill domèstic (*Acheta domesticus*) [50] com a nous aliments.

D'altra banda, la Comissió Europea va autoritzar l'ús d'insectes com a pinsos, inicialment per a l'aqüicultura el 2017, i posteriorment per a porcs i aus de corral el 2021 [51]. Tradicionalment, els pinsos comercials per al bestiar es componen de grans de pinso, com el blat de moro, la soja, la melca, la civada i l'ordi, constituint aproximadament un terç de tots els cereals produïts. Els pinsos a base d'insectes representen una oportunitat per substituir els de cereals. Cal destacar que hi ha un interès creixent per incorporar proteïna d'insectes en les formulacions d'aliments per a mascotes, sent les larves de mosca soldat negra les més utilitzades en aquest sector [52,53]. A més, entre els candidats més prometedors i més estudiats per a la producció industrial de pinsos es troben cucs de farina grocs, cucs de seda, saltamartins i tèrmits [54].

### 2.3.1. Cucs de farina per l'alimentació humana i animal

*Tenebrio molitor* i *Alphitobius diaperinus* són formes larvàries de diferents espècies d'escarabats de la família Tenebrionidae, coneguts com els escarabats foscos [55–58]. La femella pon típicament una mitjana de 400-500 ous, i l'etapa larvària dura aproximadament 6-8 mesos en condicions òptimes [59]. A més, mitjançant l'ajust de condicions específiques, es pot regular i optimitzar el seu cicle de vida per garantir una producció massiva eficient [60]. Com l'ús d'insectes, la seguretat i el contingut nutricional varia segons l'etapa del cicle vital en què es consumeixin [61], cal destacar que es proposen i s'utilitzen principalment en forma de derivats de cucs crus o enriquits amb proteïnes (farines d'insectes) en matèria seca.

Amb gairebé dos mil milions de persones a tot el món consumint insectes, els cucs de la farina, que sovint es troben en la farina i grans de cereals, també s'utilitzen per al consum humà [32], i a la Xina, el cuc groc de la farina és un plat popular [62]. Ambdues larves comestibles han estat avaluades positivament per l'EFSA i tenen un perfil nutricional similar: són riques en proteïnes i tot l'espectre d'aminoàcids, riques en àcids grassos essencials i vitamines, i més altes en certs minerals (particularment calci, coure, magnesi, ferro i zinc) que la carn i els ous convencionals [63,64].

A més, els cucs de la farina s'han aplicat per a l'alimentació en diferents sectors animals i és un dels insectes més estudiats. D'una banda, el 2015, Bovera *et al.* va demostrar la viabilitat de substituir la farina de soja per farina de larves de *T. molitor* en dietes de pollastre d'engreix, en termes d'ingesta d'aliment i taxa de creixement, amb major ràtio de conversió d'aliment i efectes prebiòtics en pollastres d'engreix amb consum de *T. molitor* [65]. Així mateix, el mateix autor va realitzar un experiment paral·lel durant el període de creixement dels pollastres d'engreix, i de nou, va demostrar els efectes més saludables d'aquest insecte quan s'utilitza com a principal contribuent proteic a la dieta [66]. En l'alimentació aquàtica, *T. molitor* també s'ha considerat com a possible font alternativa de proteïnes per substituir la farina de peix [67]. Fins i tot, un estudi va descriure que també es pot utilitzar en un sistema de suport vital bioregeneratiu a l'espai, proporcionant un sistema eficient per tractar els residus vegetals i proposant-la com a proteïna d'alta qualitat per als astronautes [62]. A més, s'ha demostrat que *T. molitor* té un alt valor nutritiu i una considerable activitat bioactiva en animals i humans [29,68]. D'altra banda, *A. diaperinus*, també conegut com cuc de la farina Buffalo, també presenta proteïnes d'alta qualitat i un perfil d'àcids grassos equilibrat [69]. No obstant això, aquest insecte no s'ha utilitzat tant per alimentar animals ni estudiat *in vivo* com el cuc groc de la farina. En aquest sentit, més estudis podrien millorar la seva aplicació en diferents sectors.

Finalment, la integració dels insectes i, en particular, dels cucs de la farina en la producció d'aliments i pinsos està en línia amb els esforços per promoure la sostenibilitat i reduir l'impacte ambiental i ofereix noves solucions als reptes alimentaris globals. No obstant això, malgrat el potencial d'acceptació creixent i suport normatiu per al consum d'insectes, hi ha preocupacions sobre la possible reacció al·lèrgica, que requereix més estudis i regulacions en profunditat per garantir la seguretat dels consumidors.

### 2.3.2. Al·lergenicitat als insectes com a aliment

La prevalença de l'al·lèrgia alimentària en els països occidentals és de fins a un 6% i segueix augmentant, pel que representa un important problema de salut [70,71]. Les al·lèrgies alimentàries són reaccions adverses a antígens alimentaris específics, que típicament són inofensius per a la població general però desencadenen mecanismes immunològics en individus susceptibles [72]. En aquest sentit, el consum d'insectes planteja preocupacions sobre les respostes al·lergògenes en individus sensibilitzats o al·lèrgics a les proteïnes de crustacis, cacauets o àcars de la pols domèstica (HDM) [73]. Dins de la família dels artròpodes, s'han identificat diversos al·lèrgens, incloent la tropomiosina [74], l'arginina-cinasa [75] i el glutatió S-transferasa [76], que exhibeixen reactivitat creuada en humans.

De fet, Broekman *et al.* va trobar que 13 de cada 15 pacients al·lèrgics confirmats a les gambes van mostrar reaccions al·lèrgiques a *T. molitor* . L'exposició a fraccions de la proteïna del cuc groc de la farina va revelar la reactivitat creuada de la IgE en aquests pacients, amb la tropomiosina i l'arginina-cinasa identificades com els principals al·lèrgens reactius creuats en el cuc de la farina groga [77]. A més, es van identificar la tropomiosina, l'α-amilasa i la miosina muscular de tres cucs de la farina comestibles per reaccionar creuadament amb sèrums de HDM (n = 11) i pacients al·lèrgics a crustacis (n = 8) [78]. Un altre estudi realitzat pels mateixos autors va introduir altres insectes (*Z. morio*, *A. diaperinus*, *G. mellonella*, *H. illucens, A. domesticus* i *Locusta migratoria*) als participants i va concloure una propensió a les reaccions al·lèrgiques no només a *T. molitor* però també a altres insectes [79]. La tropomiosina, una vegada més, va emergir com un al·lergen creuat clau per als pacients al·lèrgics a HDM i gambes; així com les proteïnes de la cutícula larvària que semblaven jugar un paper important en la reactivitat creuada dels pacients principalment sensibilitzats al cuc de la farina [80]. D'altra banda, la tropomiosina de *G. bimaculatus* ha descrit que indueix una reacció al·lèrgica en individus amb al·lèrgia confirmada als crustacis [81].

A més, un informe de cas de França va detallar una anafilaxi alimentària severa induïda per larves cuites de *T. molitor* en un home de 31 anys al·lèrgic a HDM però no a crustacis. Les proteïnes al·lèrgiques responsables d'aquesta sensibilització incloïen l'hexamerina, la tropomiosina, l'α-amilasa i les proteïnes de cutícula larvària A1A i A2B [82].

En canvi, en models de rosegadors sans, les llargues exposicions orals de rates Sprague-Dawley, a dosis de 300-3000 mg/kg/dia de cuc de la farina no van augmentar significativament els biomarcadors al·lergògens com la histamina o les concentracions d'IgE en sèrum [83].

No obstant això, s'ha explorat mitigar o fins i tot eliminar al·lèrgens dels insectes durant el seu processament mitjançant mètodes com el tractament tèrmic i la hidròlisi enzimàtica [84,85]. Altres estudis científics en aquesta direcció estan en curs i ofereixen solucions valuoses per reduir l'al·lergenicitat de les formulacions que incorporen proteïnes d'insectes.

## 2.4. Valor nutricional dels insectes

Els insectes comestibles es caracteritzen per un excel·lent perfil nutricional, posicionant-los com una font alternativa de proteïnes viable tant per a humans com per a animals.

En l'etapa larvària, els insectes tenen un alt percentatge de proteïnes i, en algunes espècies, arriben fins al 60% del total de nutrients en termes de pes sec (taula 1) [86]. A més, la proteïna dels insectes conté tots els aminoàcids essencials (AAE) i s'ha descrit que n'aporta nivells superiors als diaris recomanats per a adults [87]. Més enllà del seu contingut proteic, els insectes comestibles exhibeixen nivells elevats d'àcids grassos insaturats, inclosos els greixos monoinsaturats (de les sigles en l'anglès, MUFA) i poliinsaturats (de l'anglès, PUFA), així com dipòsits rics en minerals, vitamines i fibra [88,89]. La seva riquesa nutricional s'estén a diversos micronutrients com el coure, ferro, magnesi, manganès, fòsfor, seleni i zinc, juntament amb vitamines essencials com riboflavina, àcid pantotènic, biotina i, en alguns casos, àcid fòlic, posicionant els insectes com una excel·lent font de nutrients [90]. A més, estudis recents apunten que podrien proporcionar compostos bioactius, com els compostos fenòlics i flavonoides [91]. Cal destacar que la composició de nutrients dels insectes està molt influenciada pel seu pinso, cosa que obre oportunitats per a la regulació, l'enriquiment i l'addició d'ingredients alimentaris específics [61].

**Taula 1.** Composició nutricional dels insectes, Tenebrio molitor and Alphitobius diaperinus, ametlla (com a font de proteïna vegetal), i vedella (com a font convencional) [47,92,93]. SFA: àcids grassos saturats; MUFA: àcids grassos monoinsaturats; PUFA: àcids grassos poliinsaturats.

| Valor nutricional (100g de producte) | *Tenebrio molitor* | *Alphitobius diaperinus* | Ametlla | Vedella |
|---|---|---|---|---|
| Energia (kcal) | 505 | 510 | 579 | 198 |
| Proteïna (g) | **57.2** | **59.6** | **21.2** | **19.4** |
| Greixos totals (g) | 28.4 | 28.7 | 49.9 | 12.7 |
| SFA (g) | 6.9 | 10.1 | 3.5 | 5.3 |
| MUFA (g) | 14.4 | 9.7 | 31.6 | 4.8 |
| PUFA (g) | 7.0 | 8.8 | 12.3 | 0.5 |
| Carbohidrats (g) | 1.8 | 2.7 | 21.6 | 0.0 |
| Sucres (g) | 0.2 | 0.5 | 3.6 | 0.0 |
| Fibra (g) | 6.4 | 7.74 | 12.5 | 0.0 |

Els aliments convencionals d'origen animal (ous, llet i carn), així com els aliments rics en proteïnes d'origen vegetal (soja) es consideren fonts proteiques d'alta qualitat perquè compleixen els requisits d'aminoàcids essencials o indispensables, i són fàcilment digerits i absorbits per l'intestí prim de mamífers [94]. En canvi, diverses fonts vegetals de proteïnes (cereals, mongetes, llegums o verdures) manquen d'alguns AAE, i la digestibilitat proteica és menor que en els insectes comestibles [95]. Quan es comparen els insectes amb totes aquestes fonts proteiques, els estudis suggereixen que el contingut de proteïnes i AAE dels insectes és comparable als aliments convencionals d'alta qualitat animal i vegetal [96], i presenten un major valor energètic, àcids grassos insaturats i certes vitamines, però menys àcids grassos saturats que la carn. A més, contràriament a la carn, els insectes també són font de vitamina C i fibra dietètica [97]. El contingut proteic en insectes comestibles comuns com *Acheta domesticus* (72,45%), *Tenebrio molitor* (58%) i *Antharaea assamensis pupae* (38,05%) és superior al de llegums com les llenties (26,7%), les mongetes (23,5%) i la soja (41,1%) [98].

La digestibilitat dels insectes és variable i es veu afectada per la presència de quitina [99], que no es degrada ni s'absorbeix a l'intestí prim encara que és superior en aliments vegetals. Estudis recents apunten a la necessitat d'augmentar i millorar les avaluacions de la digestibilitat dels aminoàcids ileals i la síntesi de proteïnes postprandials *in vivo*, per avaluar i comparar adequadament la qualitat de les proteïnes derivades d'insectes amb altres fonts proteiques [96]. En aquest sentit, Lanng *et al.* descriu una major quantitat d'aspartat, metionina, glutamat, aminoàcids de cadena ramificada (BCAA) i els aminoàcids aromàtics, tirosina i fenilalanina, que arriben a l'intestí prim, després de la ingestió d'insectes en comparació amb la ingestió de carn de porc [100]. A més, Dai *et al.* també va demostrar un major nivell de leucina, BCAA i EAA després del consum de proteïna de grill, mentre que la concentració d'aminoàcids no essencials i aminoàcids totals va ser major per a la proteïna derivada de la carn de boví [101].

Tenint en compte l'evidència existent, els insectes s'han postulat com una font potencial de proteïnes d'alta qualitat, en termes de contingut d'aminoàcids essencials i digestibilitat. Així, els insectes es troben entre els futurs aliments suggerits per proporcionar alternatives de bona qualitat als aliments actuals d'origen proteic [26].

### 2.4.1. Salut més enllà de la nutrició: bioactivitat dels insectes comestibles

Com s'ha esmentat anteriorment, els insectes són una font de compostos bioactius, que poden tenir un efecte beneficiós sobre la salut quan es consumeixen. Exemples d'aquests compostos que es poden trobar en els insectes són pèptids, compostos fenòlics, la quitina i el quitosan [102–105]. Els pèptids bioactius consten de 2 a 20 aminoàcids com a seqüència no activa de proteïnes, obtinguda a partir de la hidròlisi enzimàtica, la digestió *in vivo* o *in vitro*, la fermentació microbiana i altres tecnologies de processament [106,107]. La quitina i el seu derivat, quitosan, són polisacàrids altament presents en els insectes, perquè és el principal material orgànic de l'esquelet de l'artròpode [102]. Per la seva banda, un gran nombre de polifenols identificats en insectes solen ser sintetitzats per les plantes hostes abans de ser metabolitzats o absorbits pels propis insectes [91]. S'ha demostrat que la bioactivitat d'aquests compostos genera diversos beneficis per a la salut.

Per totes aquestes raons, hi ha un interès creixent en les potencials propietats bioactives dels components dels insectes i els seus hidrolitzats proteics [29,44], incloent propietats antioxidants, antimicrobianes, antihipertensives i antidiabètiques, que podrien aplicar-se en aliments funcionals o nutracèutics [98].

#### Efectes sobre el metabolisme i la ingesta d'aliments

La bioactivitat dels insectes s'ha suggerit en termes de millora del metabolisme i control del pes. D'una banda, alguns estudis han demostrat la inhibició de la DPP-IV, enzim responsable de la reducció de la insulina i, en conseqüència, de l'augment de glucosa en sang postprandial, per *Gryllodes sigillatus* o *Antheraea assamensis* hidrolitzats, suggerint propietats antidiabètiques [108,109]. A més, estudis *in vivo* van mostrar l'eficàcia d'extractes etanòlics de cucs de seda i glicosaminoglicans de grills per millorar el metabolisme de la glucosa i l'efecte antioxidatiu en ratolins diabètics tipus 2 [110,111]. La suplementació crònica amb *larves de Proteatia brevitaris* durant set setmanes millora l'estat de salut dels ratolins alimentats amb una dieta alta en greixos (HFD, de l'anglès), en reduir l'augment de pes corporal (PC), el pes de greix epidídim i subcutani, i l'acumulació de lípids en els nivells hepàtics i plasmàtics [112]. Un altre estudi amb extractes etanòlics de *Gryllus bimaculatus* en rates alimentades amb dieta alta en greixos, contraresten el dany induït per la dieta amb una reducció del pes gras abdominal i epidídim, els nivells de colesterol i triglicèrids, suggerint el seu potencial antiateroscleròtic o antiinflamatori [113]. No obstant això, pel que fa als efectes sobre PC, no hi ha consens sobre la ingesta de *T. molitor* en models animals. Les dosis altes de *suplements de T. molitor* van mostrar una tendència a augmentar el PC i el consum d'aliments en rates mascles [114]. En rates obeses, la incorporació d'aquest insecte a la dieta va provocar efectes lipídics reductors en el fetge i el plasma [115]. Per contra, alguns autors no van reportar cap efecte en el PC o la ingesta d'aliments després de tractaments crònics en rates mascles [116] o femelles [83]; mentre que altres van indicar que les dietes basades en cucs van conduir a una reducció de l'augment de pes i una millora del metabolisme en ratolins en situació d’obesitat induïda per la dieta [58]. A més, en un estudi previ del nostre grup de recerca, l’administració d’*A. diaperinus* va modificar la secreció entero hormonal intestinal *ex vivo* i es va associar a un augment de la ingesta d'aliments [117].

### Efectes antihipertensius

Es suggereix principalment que les propietats antihipertensives s'aconsegueixen a través de la inhibició de l'enzim convertidor de l'angiotensina I [118]. Sousa *et al.* va demostrar la capacitat dels hidrolitzats de proteïnes d'*Alphitobius diaperinus* per inhibir eficaçment aquest enzim [119]. *In vivo*, es va descriure que *Tenebrio molitor* pot reduir la pressió arterial, la freqüència cardíaca i la pressió de perfusió coronària de rates espontàniament hipertensives [116].

### Propietats antioxidants i immunomoduladores

Un estudi va determinar que la capacitat antioxidant *in vitro* era 5 vegades superior a la del suc de taronja natural, per a diferents extractes de saltamartins, cucs de seda i grills [120]. Zielinska *et al.* va demostrar que el contingut fenòlic total i la capacitat antioxidant, mesurada com la capacitat de neutralitzar ABTS·+ i DPPH·, augmentava quan també s’augmentava el percentatge de farina d'insecte afegida en magdalenes [121]. Aquesta propietat es va associar al contingut fenòlic però també als pèptids obtinguts dels hidrolitzats de proteïnes [118]. Un anàlisi *in silico* va proposar que alguns pèptids amb activitat antiinflamatòria es podien trobar en *T. molitor* [122]. En aquest sentit, un altre treball va demostrar que els preparats proteics derivats de la digestió *in vitro* d'insectes (*G. sigillatus*, *T. molitor*, i *S. gragaria*) podrien ser fonts de pèptids bioactius amb efectes antioxidants i antiinflamatoris, accentuant-se després del procés de tractament tèrmic, que millora significativament aquestes propietats [123]. L'estudi també va demostrar que els hidrolitzats de proteïnes exerceixen efecte antiinflamatori mitjançant la inhibició de lipoxigenases (LOX) i ciclooxigenases (COX). Tot i que aquesta propietat dels insectes no s'ha investigat àmpliament, un estudi va informar que el consum de grills durant 14 dies en adults sans, va reduir la inflamació sistèmica disminuint els nivells plasmàtics de TNF-α i va millorar la salut intestinal augmentant el creixement de bacteris probiòtics [124]. D'altra banda, Park *et al.* va informar de l'efecte protector dels components bioactius d'*A. dichotoma* en la integritat de la barrera i les respostes inflamatòries en model amb inflamació vascular induïda per lipopolisacàrids (LPS) [125]. A més, pèptids antimicrobians com les defensines, cecropins i melittina, que són components essencials del sistema immunitari innat, podrien obtenir-se d'algunes espècies d'insectes [126]. Per últim, se sap que el quitosan present en insectes actua com a agent quelant en sistemes biològics i té activitat antimicrobiana contra bacteris, llevats i fongs [127].

### Efectes sobre la salut intestinal

Alguns estudis també han analitzat específicament els efectes del consum d'insectes en el tracte intestinal. Per exemple, es va descriure que *G. bimaculatus* suprimeix l'estrès oxidatiu intestinal i mitiga les respostes inflamatòries a l'intestí prim, protegint-lo de la hiperpermeabilitat induïda pel consum d'alcohol en ratolins [128]. Un altre estudi va informar de la reducció de l'expressió gènica d'IL-1β, IL-6 i TNF-α, així com l'atenuació dels canvis patològics en el còlon induïts per DSS en ratolins, quan s'alimentaven amb pols de larves de *Tenebrio molitor* [129].

En un treball recent, la substitució parcial i total de la farina de peix per farines d'*A. diaperinus* i *T. molitor* va mostrar un creixement saludable, una composició saludable del microbioma i una expressió gènica immune saludable en els salmons atlàntics [130]. A més, Ge *et al.* va proposar que una dieta que incorpora cucs de farina grocs millora la funció immunitària intestinal en un model de dany intestinal de peix, proporcionant evidència de beneficis per a la salut també en la indústria de l'aqüicultura [131].

D'altra banda, tenint en compte que l'intestí és un hàbitat per a milers de bacteris que componen la microbiota, que poden metabolitzar compostos dels aliments ingerits i influir tant en la salut intestinal com en general, alguns estudis han explorat la modulació de la microbiota pel consum d'insectes. Per exemple, Young *et al.* van estudiar la fermentació de diferents larves digerides *in vitro* en cultius de lots fecals humans, i van demostrar l'augment de bacteris amb propietats antiinflamatòries i associacions amb la salut intestinal [132]. Per altra banda, un estudi va apuntar que el consum de larves d'insectes induïa una lleugera millora en la microbiota fecal de pollastres, millorant la població de bacteris productors d'àcids grassos de cadena curta (SCFA) [133]. En rates, una substitució parcial de la carn per proteïna aïllada d'*A. diaperinus* també va resultar en alteracions de la composició del microbioma, acompanyades de canvis gastrointestinals i plasmàtics del perfil de metabòlits [100]. A més, en l'estudi de Kang *et al. T. molitor* o *A. diaperinus* es van incloure en la dieta de ratolins d'obesitat induïda per la dieta i van donar lloc a canvis en el microbioma intestinal i beneficis metabòlics per a aquests animals[58]. Segons totes aquestes evidències, el consum d'insectes podria induir canvis en el microbioma d'animals i humans i, en conseqüència, en el sistema intestinal i sistèmic, que podrien estar associats a beneficis per a la salut.

Taula 2. Publicacions dels efectes de consumir insectes en models animals sobre la inflamació, salut intestinal i funció barrera.

| Espècie d'insecte | Tipus cel·lular o model animal | Health status | Efecte | Referència |
|---|---|---|---|---|
| *A. dichotoma* | *In vitro* | Cèl·lules endoteli tractades amb LPS | Protecció inflamació i disrupció barrera | Park *et al.* 2020 [125] |
| *G. sigillatus,*<br>*T. molitor,*<br>*S. gragaria* | *In vitro* | - | Inhibició de la LOX i COX | Zielińska *et al.* 2018 [123] |
| *G. sigillatus* | Humà | Sà | ↑ Creixement bacteris probiòtics<br>↓ TNF- α nivells plasmàtics | Stull *et al.* 2018 [124] |
| *T. molitor* | Ratolí | Colitis induïda per DSS | Protecció colònica<br>↓ IL-1β, IL-6, TNF-α expressió gènica | Park *et al.* 2023 [129] |
| *G. bimaculatus* | Ratolí | Hiperpermeabilitat i estrès oxidatiu induïts per l'alcohol | ↓ Estrès oxidatiu<br>↓ Inflamació | Hwang *et al.* 2019 [128] |
| *T. molitor*<br>*A. diaperinus* | Ratolí | Obesitat induïda per HFD | ↓ Expressió gens relacionats amb immunitat i apoptosis | Kang *et al.* 2023 [58] |
| *T. molitor* | Perca americana | Dany intestinal | Millora funció immune intestinal | Ge *et al.* 2023 [131] |
| *T. molitor*<br>*A. diaperinus* | Salmó Atlàntic | Sà | ↑ Expressió gens relacionats amb immunitat intestinal | Habte-Tsion *et al.* 2024 [130] |
| *T. commodus*<br>*C. giveni* | Cultius de femta humana | Sà | Millora microbiota<br>↑ Propietats antiinflamatòries i salut intestinal | Young *et al.* 2020 [132] |
| *H. illucens*<br>*T. molitor* | Pollets | Sà | = composició mucines<br>= Resposta immune local<br>Millora microbiota cecal | Colombino *et al.* 2021 [133] |
| *T. molitor* | Pollastres | Sà | ↑ Rati conversió aliments<br>↑ Allargada intestí | Bovera *et al.* 2016 [66] |

Tenint en compte totes aquestes evidències, els insectes o productes que els puguin incorporar com a ingredients, es presenten com alternatives saludables i comparables als productes carnis[89], i fonts potencials de compostos bioactius[91]. No obstant això, hi ha una manca d'estudis de la bioactivitat intestinal del consum insectes. Per tant, realitzar-ne més *in vivo,* que explorin específicament els seus beneficis per a la salut, podria servir com un factor motivador perquè les persones incloguin aquesta font de proteïna alternativa com un component habitual de la seva dieta diària.

Així doncs, l'exploració dels efectes del consum d'insectes en el tracte gastrointestinal és una àrea d'investigació prometedora per comprendre el seu impacte més ampli en la salut general. A més, es necessiten estudis a llarg termini amb diferents proporcions d'insectes i proteïnes d'insectes per dilucidar completament els seus efectes i mecanismes subjacents en el cos [1]. Aquesta recerca és essencial per augmentar el coneixement científic d'aquests nous aliments amb la finalitat d'augmentar l'acceptabilitat social dels insectes comestibles, principal barrera per a la seva introducció en les dietes occidentals.

## 3. El tracte gastrointestinal i la modulació de les seves funcions pels aliments

### 3.1. Anatomia, fisiologia i funció general de l'intestí

El tracte gastrointestinal (GI) representa la superfície més gran entre el medi extern i l'interior del cos humà [134]. L'intestí és l'òrgan responsable de la digestió i absorció de nutrients. Quan els components dels aliments arriben a l'intestí, conclou el procés digestiu iniciat a la cavitat oral. En primer lloc, a l'intestí prim, l'aigua i els nutrients de mida més petita, inclosos sucres, aminoàcids lliures i ions, s'absorbeixen a la circulació sanguínia. Posteriorment, a l'intestí gros o al còlon, s'acumulen molècules i fibres més grans per ser descompostes per la microbiota, també coneguda com fermentació de fibra, i té lloc l'absorció d'altres nutrients, com les vitamines [135]. A més, l'epiteli intestinal serveix de barrera física que separa dos compartiments oposats. En aquest sentit, més enllà del seu paper en l'absorció de nutrients, assumeix el paper en la defensa de l'organisme enfront als patògens luminals [136], i controla la resposta immunitària de la mucosa i l'activitat metabòlica de l'intestí.

El sistema gastrointestinal està connectat als sistemes vascular i limfàtic, facilitant la regulació de la ingesta d'aliments i la funció digestiva, i el lliurament de compostos ingerits als òrgans de tot el cos [137,138]. A més, està estretament connectat amb el sistema nerviós. A la paret del tracte gastrointestinal, una extensa xarxa neuronal forma el sistema nerviós entèric (ENS), que també està implicat en la mobilitat, l'activitat secretora i immunològica del tracte gastrointestinal [139]. Així mateix, també s'ha descrit un eix intestí-cervell, pel qual diversos pèptids o hormones que són secretades per l'intestí en resposta als nutrients entren a la circulació i actuen directament sobre el cervell per ajudar a mantenir l'homeòstasi [138]. Aquests vincles amb els sistemes circulatori, immunitari i nerviós subratllen l'impacte sistèmic dels processos intestinals, estenent la seva influència molt més enllà del tracte digestiu.

En els últims anys, l'intestí ha guanyat atenció pel seu paper fonamental en la fisiopatologia d'alguns trastorns de salut. D'altra banda, el coneixement existent dels efectes cel·lulars exercits pels nutrients a través de l'intestí evidencia la possibilitat de la desregulació de l'homeòstasi intestinal per alguns components de la dieta, mentre que altres poden oferir una modulació positiva [140,141]. Així doncs, la identificació i interpretació dels mecanismes pels quals els hàbits nutricionals i els compostos dietètics influeixen en la salut intestinal promovent i/o restaurant la seva homeòstasi és un important camp d'estudi que requereix un coneixement de l'estructura intestinal i les seves funcions.

### Estructura intestinal i procés de digestió

El sistema intestinal humà adult consta de dues parts: l'intestí prim, que mesura aproximadament 6 – 7 m de longitud i 2,5 - 3,0 cm de diàmetre, i l'intestí gros, que oscil·la entre 1,5 i 2 m de longitud amb un diàmetre de 6 - 7,5 cm [137,142]. L'intestí prim es divideix anatòmicament en tres regions diferents - el duodè, el jejú i l'ili-, mentre que l'intestí gros està segmentat en caecum, còlon ascendent i descendent, recte i anus.

Histològicament, la paret del tub muscular que es reconeix com a intestí està formada per diferents capes: mucosa, submucosa, *musculosa propria* i serosa. La mucosa, la capa més interna de l'intestí, està constituïda per la *lamina propria*, les mucoses musculars i l'epiteli. La *lamina propria* és una capa de teixit connectiu reticular formada per elastina, reticulina, fibres de col·lagen, limfòcits, cèl·lules plasmàtiques, granulòcits, vasos limfàtics i capil·lars. En canvi, la *musculosa mucosa* és una fina capa de múscul llis entre la mucosa i la submucosa. Ambdues capes ofereixen suport i articulen la capa de l'epiteli [143]. Finalment, la serosa és una membrana llisa formada per una fina capa de teixit connectiu i una fina capa de cèl·lules que secreten líquid serós per lubricar les estructures internes [144].

Al llarg de l'intestí, l'epiteli es plega per formar estructures com són les vellositats i les criptes (Figura 1), descrites com projeccions luminals o invaginacions que augmenten significativament l'àrea superficial [145]. A més, la superfície apical dels enteròcits està coberta per projeccions microscòpiques semblants als dits anomenades microvellositats (100 nm de diàmetre), augmentant més l'àrea d'absorció (M), conferint uns 30 m$^2$ de superfície a l'intestí prim [146].

El procés digestiu té lloc a tot l'intestí, amb funcions específiques a cada part. El quim, massa d'aliments semidigerits arriben de l'estómac al duodè, la primera part de l'intestí prim, passant d'un ambient àcid a un pH neutre i a un estat més líquid anomenat quil. Els enzims del pàncrees i els àcids biliars arriben a aquesta regió per descompondre proteïnes, greixos i hidrats de carboni, així com per emulsionar els greixos [135]. Progressant des del duodè, el jejú i l'ili constitueixen una gran superfície per permetre l'absorció de les petites molècules produïdes. En tot aquest procés, els moviments del peristaltisme són importants per exercir la barreja i el transport del quil per totes les seccions intestinals. En arribar al còlon, la microbiota que hi resideix porta a terme la fermentació dels components residuals dels hidrats de carboni que encara no han estat digerits, a causa de l'ambient anaeròbic característic del còlon [147]. A més, aquí també es produeix el trencament de les proteïnes i pèptids restants, inclosos els derivats de l'hoste (com les mucines). Els productes generats són absorbits pels enteròcits de les criptes i serveixen com a molècules de senyalització, iniciant respostes immunitàries i metabòliques sistèmiques [146,147]. Simultàniament, el quil es torna menys líquid, a causa de l'absorció d'aigua i de minerals, i s'emmagatzema i compacta en el còlon descendent abans d'una eventual defecació.

### Tipus cel·lulars

El tracte gastrointestinal està compost per diferents tipus de cèl·lules epitelials en la línia epitelial. A la base de les criptes hi ha cèl·lules mare indiferenciades [135], que es desenvolupen en tipus de cèl·lules epitelials funcionals: enteròcits o colonòcits, cèl·lules de Paneth, cèl·lules de Tuft, cèl·lules caliciformes (anomenades Goblet) i cèl·lules enteroendocrines [145] (Figura 1). Aquestes cèl·lules regeneren contínuament l'epiteli (cada 3-5 dies) mentre migren per l'eix cripta-vellositats abans de sotmetre's a una apoptosi programada [148]. Cada tipus madura segons la seva funció específica i migra amunt i fora de la cripta per tot l'intestí; No obstant això, la distribució de cada tipus cel·lular varia segons la funció específica de cada regió [149].

Les cèl·lules epitelials intestinals formen una barrera física, ja que són impermeables al contingut luminal [150]. Els enteròcits, el tipus cel·lular més abundant, i les cèl·lules enteroendocrines es troben per tot l'intestí i estan implicats en l'absorció de nutrients i aigua, i la producció d'hormones, respectivament [151]. Les cèl·lules caliciformes, que es troben en major nombre a l'intestí gros, són les encarregades de produir mucus; mentre que les cèl·lules de Paneth resideixen a l'intestí prim i contribueixen a la immunitat innata secretant pèptids antimicrobians, proteïnes i altres components que són importants en la defensa i immunitat de l'hoste [152]. Les cèl·lules Tuft són cèl·lules quimiosensorials, que recentment s'han descrit també com a cèl·lules secretores que inicien la resposta immunitària tipus 2, jugant un paper crucial en la detecció d'infeccions parasitàries o al·lèrgens i desencadenant la reacció immunitària adequada [153]. Finalment, en zones específiques de l'epiteli anomenades plaques de Peyer, hi ha cèl·lules especialitzades anomenades cèl·lules M, que són capaces de transportar antígens luminals i bacteris, i presentar-los als limfòcits que activen o inhibeixen la resposta immune [154].

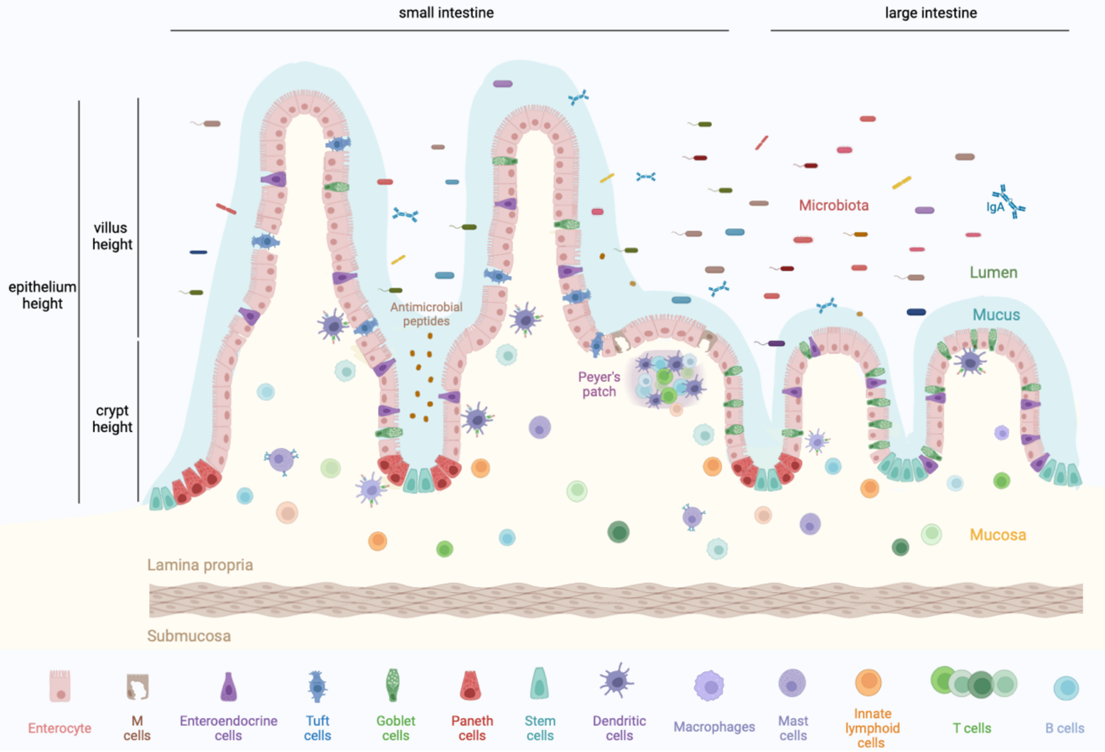

**Figura 1.** Estructura i composició intestinal. Diferents estructures de l'intestí prim i gruixut, i la morfologia de l'epiteli. Capes de la barrera intestinal que s'estenen des del lumen fins a la submucosa. Al lumen s'hi troben components com la microbiota intestinal, els pèptids antimicrobians, IgA i els compostos derivats de la microbiota com l'LPS. La capa de mucus actua com a barrera, impedint el contacte directe entre la microbiota intestinal i les cèl·lules epitelials. La capa cel·lular epitelial consisteix principalment en enteròcits, al costat d'altres tipus de cèl·lules secretores, incloent-hi enteroendocrina, cèl·lules tuft, i cèl·lules goblet i Paneth. Diversos tipus de cèl·lules immunitàries poblen la làmina pròpia, servint per protegir el cos contra antígens i bacteris, prevenint així la inflamació sistèmica. Creat amb BioRender.com.

Sota la monocapa de cèl·lules epitelials, *la lamina propria* està constituïda per poblacions molt diverses de cèl·lules, incloent cèl·lules immunitàries com limfòcits B i T, cèl·lules dendrítiques (CD), macròfags, eosinòfils, neutròfils i mastòcits, entre d'altres [155]. Les CD es localitzen principalment en contacte amb l'epiteli a l'intestí prim, però també es poden trobar al gruixut en resposta a estímuls inflamatoris [151]. Posseeixen la capacitat de capturar, processar i presentar antígens a limfòcits T naïfs, contribuint a la resposta immunitària i la tolerància [156]. En canvi, els macròfags, àmpliament presents en tota la longitud de l'intestí, capturen i maten microbis, fagociten material apoptòtic i cèl·lules mortes, i secreten citocines [157]. Els neutròfils, d'altra banda, juguen papers tant en la iniciació com en la resolució de la inflamació, contribuint a l'activitat fagocítica i reclutant altres cèl·lules immunitàries mitjançant l'alliberament de citocines [158]. Els eosinòfils, localitzats principalment al duodè, són granulòcits implicats en l'eliminació de paràsits, la regulació de les respostes de les cèl·lules T, la promoció de la reparació de teixits i la contribució a respostes al·lèrgiques [159,160]. Els mastòcits, en constant interacció amb els eosinòfils, són fonamentals en el desenvolupament de la resposta al·lèrgica alliberant histamina i mediadors inflamatoris, influint així en les funcions d'altres cèl·lules immunitàries [161]. Finalment, els limfòcits T i B estan implicats en la resposta adaptativa immune amb papers crítics en la vigilància immunitària, tolerància i defensa mitjançant la generació de cèl·lules efectores i anticossos específics d'antigen per neutralitzar i eliminar patògens [162].

En conjunt, les cèl·lules de l'epiteli intestinal i els factors secretats, juntament amb la diversa gamma de cèl·lules immunitàries presents a la *lamina propria*, formen la barrera intestinal [134], un concepte que s'explica en profunditat en el següent apartat.

## 3.2. Barrera intestinal en salut i malaltia

### 3.2.1. Homeòstasi de la barrera intestinal

La barrera intestinal fa referència al complex sistema encarregat de mantenir un delicat equilibri entre absorció i protecció. Formada per la microbiota, la capa epitelial, el mucus, pèptids antimicrobians i cèl·lules immunitàries, la barrera intestinal actua com a primera línia de defensa contra patògens i substàncies nocives presents en el lumen intestinal [163]. D'aquesta manera, la barrera integra els factors físics, bioquímics i immunològics, un sistema que orquestra un mecanisme de defensa multifacètic per mantenir la salut intestinal i general.

#### Microbiota intestinal

La part luminal de la mucosa conté uns 100 bilions de microorganismes, coneguda com la microbiota intestinal. En general, està composta per bacteris de 6 fílums, entre els quals *Firmicutes* i *Bacteroidetes* són els principals tipus, però també contenen fongs, virus, i arqueus [164]. Durant l'embaràs, amb els bacteris presents a la placenta i a l'entorn uterí, i principalment en néixer, es dona la primera introducció de la microbiota a l'organisme [165,166]. Després, molts factors neonatals i postnatals, com la lactància materna, també juguen un paper crucial en l'establiment de la microbiota i el sistema immunitari del lactant [167]. Així, la microbiota és única entre individus i pot variar dins d'un mateix individu, a causa de l'edat, factors ambientals i dietètics [168]. A més, varia en contingut i quantitat en les diferents parts anatòmiques del tracte gastrointestinal; però es troba principalment al còlon, on el flux més lent del trànsit intestinal i l'ambient (pH, temperatura i nivells d'oxigen) afavoreixen el creixement dels tipus anaeròbics observats [168,169].

La microbiota intestinal i les cèl·lules intestinals existeixen principalment en una relació simbiòtica, en la qual els bacteris contribueixen a la immunitat, fisiologia i metabolisme de l'hoste, mentre que l'intestí proporciona un entorn ric en nutrients per als microbis [170]. El metabolisme de polisacàrids i proteïnes específiques podria requerir múltiples enzims i rutes bioquímiques produïdes per la microbiota. S'han identificat 3,3 x $10^6$ gens microbians intestinals, superant en nombre els gens codificants de proteïnes humanes en un factor de 150 [171]. En aquest sentit, un dels principals beneficis d'una microbiota intestinal "sana" és la fermentació colònica de la fibra dietètica, que resulta en la producció de diversos metabòlits, entre ells SCFAs, amoníac, amines i compostos fenòlics [172]. La majoria dels SCFA són àcid acètic, àcid butíric i àcid propiònic i tenen efectes beneficiosos ben documentats sobre la salut intestinal i sistèmica [150]. Juguen un paper crucial en les respostes metabòliques i endocrines, actuant directament com a font d'energia per a les cèl·lules hoste, però també estimulant la producció d'hormones intestinals i activant la senyalització al cervell per regular la ingesta d'aliments [170]. A més, la microbiota intestinal també té un paper clau en el manteniment de la integritat i funció de la barrera. Els SCFA posseeixen propietats antiinflamatòries i immunomoduladores [172], milloren la producció de mucus [173], regulen la secreció d'interleucina-18 (IL-18) per part de les cèl·lules epitelials i immunitàries [174], la secreció d'immunoglobulina A (sIgA) [175], i reforcen la barrera intestinal actuant sobre les unions estretes [176]. A més, altres metabòlits bacterians, com certs conjugats secundaris d'àcids biliars i metabòlits triptòfans, indueixen el desenvolupament de cèl·lules T reguladores del còlon (Treg) [150]. Col·lectivament, els metabòlits intestinals juguen un paper local crític en el manteniment de l'homeòstasi intestinal i la regulació immune, alhora que exerceixen efectes sistèmics sobre la salut general [177].

### Mucus i barrera epitelial

El mucus proporciona la primera protecció contra els microorganismes luminals [178], en limitar el contacte dels microorganismes amb l'epiteli, reduint així el risc d'invasió i infecció [152]. El mucus està format principalment per mucines secretades per cèl·lules caliciformes, particularment MUC2, una gran proteïna d'alta glicosilat que confereix la propietat gelatinosa a la mucositat [179]. Aquesta densa capa també conté els pèptids antimicrobians secretats per les cèl·lules de Paneth, que inclouen defensines, lisozim, quimiocines, etc [180]. A més, la sIgA alliberada pels limfòcits B, podria unir el mucus i ancorar els bacteris, contribuint a l'aglutinació bacteriana i facilitant la seva contenció a la capa de moc. Així mateix, aquest moc també proporciona una rica capa d'hidrats de carboni per a la microbiota de creixement lent [181].

A continuació, les cèl·lules epitelials monocapa actuen com la barrera física regulant el pas de nutrients i aigua a la circulació, però evitant l'entrada de substàncies nocives, inclosos patògens, toxines i microbiota, a l'organisme [182]. Aquesta permeabilitat selectiva està intervinguda per dues vies principals: les vies transepitelials/transcel·lulars i paracel·lulars. Les transcel·lulars consisteixen en el transport de les substàncies predominantment regulat per transportadors selectius, mentre que les paracel·lulars s'associen amb el transport que es dona en l'espai entre cèl·lules epitelials [183]. La integritat i les propietats de permeabilitat paracel·lular de l'epiteli estan regulades per diversos tipus d'estructures adhesives anomenades unions [184]. Principalment, les proteïnes transmembrana més apicals, conegudes com unió estreta (TJ), juguen un paper crucial en el control del contacte cel·lular i regulen la permeabilitat selectiva d'aquesta capa, mantenint l'homeòstasi intestinal [185]. A més, les cèl·lules epitelials inclouen cèl·lules caliciformes i de Paneth que estan enfortint constantment la barrera mucosa mitjançant la producció de mucus i AMPs, respectivament; així com cèl·lules M, conegudes per transportar els microorganismes, i molècules macro i solubles des del lumen intestinal fins a la regió subepitelial per iniciar la resposta immunitària de la mucosa [186].

### Barrera immunològica

Per sota de l'epiteli intestinal es troba la barrera immunològica de la mucosa intestinal, coneguda com a sistema immunitari de les mucoses, que constitueix l'òrgan immunitari més gran del cos humà (Figura 2). Dins d'aquest sistema, diversos tipus de cèl·lules immunitàries es troben i es distribueixen en llocs immunes inductius i efectors. D'una banda, CD, macròfags, neutròfils, mastòcits, així com cèl·lules limfoides innates (ILC),[183] limfòcits T CD4+ (Th1, Th2, Th17, cèl·lules T reguladores), cèl·lules T CD8+ i cèl·lules plasmàtiques secretores d'IgA, que existeixen difusament a la *lamina propria* i conformen el lloc efector immunitari. En canvi, els llocs immunoinductius, com el teixit limfoide associat a l'intestí (GALT), abasten estructures limfoides organitzades que inclouen fol·licles de cèl·lules B amb centres germinals envoltats, per una zona de cèl·lules T [186]. En conjunt, aquestes cèl·lules i llocs, juntament amb els altres components de la barrera, treballen conjuntament en la defensa contra la infecció per patògens i en el manteniment de la barrera intestinal [3,150].

En primer lloc, els components del sistema immunitari innat, així com les cèl·lules epitelials, utilitzen receptors de reconeixement de patrons, com els receptors tipus Toll (TLR), per discernir entre components microbians patògens i antígens (Ags) inofensius. Aquests receptors reconeixen patrons moleculars associats a microbis (MAMPs) que són estructures moleculars essencials per a la supervivència microbiana o patrons moleculars associats al dany, alliberats de cèl·lules hoste que s'enfronten a lesions o estrès molecular [187]. La seva activació indueix diverses vies intracel·lulars que resulten en la fagocitosi i/o desencadenen l'alliberament específic de citocines i quimiocines, i la secreció d'AMPs, iniciant una ràpida resposta immunitària proinflamatòria i protectora [185]. Els neutròfils actuen en la resposta immunitària innata primerenca, fagocitant microbis i produint factors microbicides i interleucines (IL). Mitjançant la producció de proteïnes granulars, com la mieloperoxidasa (MPO), contribueixen a catalitzar la formació d'espècies reactives d'oxigen (ROS) que ajuden a matar directament els patògens [158]. Un cop finalitzades les seves tasques, els neutròfils se sotmeten a una apoptosi, i els macròfags els eliminen per resoldre la inflamació i la recuperació de la funció dels teixits. A més, els macròfags i les CD, com a fagòcits mononuclears, engoleixen i eliminen els bacteris patògens alhora que secreten mediadors immunes per a la comunicació cèl·lula-cèl·lula [177]. Les ILC, per la seva banda, no tenen activitat citotòxica directa sinó que s'han descrit per secretar citocines com IL-22 que promouen la mucositat i la producció d'AMPs [183].

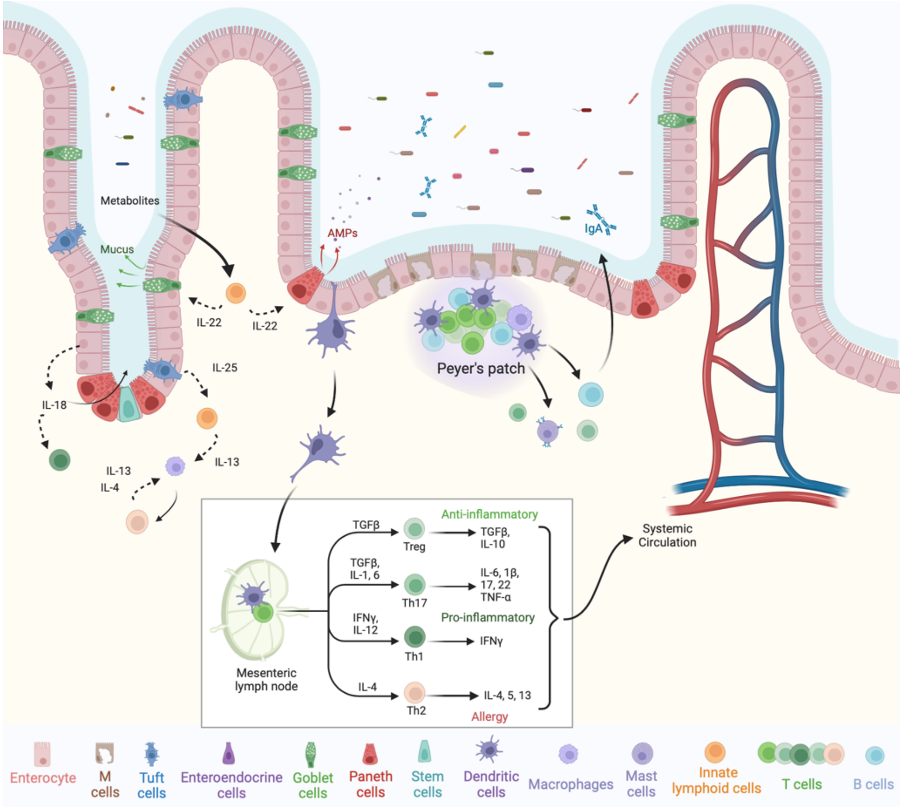

**Figura 2**. Funció barrera intestinal. Activació de cèl·lules caliciformes i de Paneth per produir mucus i AMPs, respectivament. Secreció d'IgA per activació de limfòcits T i B de les plaques de Peyer. CDs com a cèl·lula capturant antigen i presentació en estructures MALT per desencadenar la resposta immune específica. Creat amb BioRender.com.

Els CD intestinals i els macròfags són heterogenis i capaços de generar respostes tant per part de limfòcits T reguladors com efectors, en resposta al reconeixement microbià o l'Ag. Per exemple, les CD subepitelials actuen com a sentinelles immunes, capturant i processant antígens i migrant als ganglis limfàtics mesentèrics (MLN) per iniciar una resposta immunitària adaptativa activant limfòcits T naïfs, produint (via Th1 o Th17) o suprimint (via Treg) la inflamació [188]. D'altra banda, els llocs inductius de vegades existeixen en formes agregades com plaques de Peyer a l'intestí prim i fol·licles limfoides aïllats (ILF) a l'intestí prim i gros [154]. Col·lectivament, formen el GALT, responsable de respostes immunitàries específiques als antígens luminals i de la producció d'immunoglobulina A (IgA) per cèl·lules plasmàtiques diferenciades [189]. El GALT està format per l'àrea interfol·licular i l'epiteli associat al fol·licle, que consisteixen en cèl·lules M, i cèl·lules presentadores d'antigen, com les CD, que també expressen algunes unions estretes i els permeten penetrar a la capa epitelial [190]. Aquestes cèl·lules capten els antígens, els presenten als limfòcits T i B, i produeixen factors proinflamatoris, com TNF-$\alpha$, IL-1$\beta$ o IL-6, induint la diferenciació Th17 [191]. No obstant això, en resposta a la microbiota, els macròfags també produeixen IL-10, mentre que les CD produeixen TGF-$\beta$ i àcid retinoic. Aquests compostos regulen la producció de citocines proinflamatòries i el desenvolupament de Tregs, que segreguen més IL-10 i ajuden a mantenir l'homeòstasi immune suprimint respostes immunitàries excessives a antígens inofensius, inclosos bacteris comensals i proteïnes dietètiques mitjançant un mecanisme conegut com tolerància [192]. Aquest mecanisme és crucial per prevenir la inflamació inadequada a l'intestí. Així, s'ha descrit que les CD i els macròfags tenen la capacitat d'impulsar la diferenciació de cèl·lules Treg que estan implicades en la tolerància a antígens orals solubles i bacteris comensals i la capacitat de proporcionar senyals directes per a la diferenciació dels limfòcits B productors d'IgA [156].

L'activació de les cèl·lules Th17 i Treg promou el canvi de classe dels limfòcits B i la secreció d'anticossos secretors (s)IgA [162], de manera dependent dels limfòcits T. Posteriorment, les cèl·lules T i B activades migren de GALT i MLN a sang perifèrica (Figura 2). Cal destacar que l'intestí conté la major concentració de cèl·lules productores d'IgA, que secreten al dia diversos grams d'anticossos al lumen [193]. A més, la sIgA podria produir-se de manera independent dels limfòcits T contra els bacteris comensals, principalment per factors produïts per CD, o IL-10, àcid retinoic i proteïna inductora d'IgA, entre d'altres [175]. La transcitosi d'anticossos IgA dimèrics a través de les cèl·lules epitelials està mediada pel receptor Ig polimèric [194]. A la mucosa, la sIgA té diferents funcions: uneix i reté bacteris a la mucositat (exclusió immune), es lliga la superfície bacteriana i a les toxines (neutralització), i facilita l'absorció d'antígens [195].

En resum, la barrera intestinal funciona de manera coordinada per regular la resposta immunitària intestinal amb l'objectiu de prevenir la infecció sistèmica i mantenir la salut general. En aquest sentit, és important saber que la dieta influeix significativament en la interacció entre la microbiota i la resposta immunitària, en proporcionar substrats per als bacteris intestinals i modular directament les cèl·lules immunitàries. Al seu torn, la funció barrera actua com a línia crítica de defensa contra patògens i al·lèrgens de la dieta, facilitant l'absorció de nutrients alhora que evita l'entrada d'antígens i microorganismes a l'organisme [185]. A més, la barrera immunològica interna permet la convivència pacífica amb els microorganismes intestinals, orquestrant respostes inflamatòries i defensives adequades davant les amenaces [196]. La disfunció de la barrera intestinal, ja sigui per un augment de la permeabilitat epitelial o per un desequilibri en la composició de la microbiota intestinal, coneguda com a disbiosi, ha estat implicada en trastorns inflamatoris i al·lèrgies alimentàries, i contribueix a la progressió de malalties com el càncer, l'obesitat, la diabetis, o trastorns neurològics, per exemple [197].

### 3.2.2. Disrupció de la funció intestinal

La integritat de la barrera i la salut de la microbiota intestinal són crucials per mantenir la salut general i prevenir trastorns inflamatoris. Diferents insults com la dieta, l'alcohol, les drogues, les toxines, l'estrès i els patògens poden influir significativament en l'equilibri del microbioma intestinal, la permeabilitat intestinal i la resposta immunitària [198,199]. La barrera intestinal funciona com un sofisticat mecanisme de defensa, amb els seus components treballant junts de manera coordinada. El component glicolític principal de la membrana externa dels bacteris gramnegatius de la microbiota són els lipopolisacàrids bacterians (LPS), que es reconeixen com endotoxines i poden desencadenar respostes inflamatòries a través de TLR [200]. Quan la permeabilitat intestinal està compromesa, es produeix un augment de la translocació bacteriana/LPS a les capes intestinals internes, donant lloc a una inflamació local i un empitjorament de la disfunció de barrera en un cercle viciós (Figura 3). La reducció de la diversitat de la microbiota també pot donar lloc a una capa de mucus més fina, promovent una major interacció entre patògens i l'epiteli [180]. En conseqüència, el sistema immunitari de la mucosa ha de respondre mitjançant l'alliberament de citocines proinflamatòries, així com el reclutament de cèl·lules immunitàries addicionals al lloc de la inflamació. La resposta inflamatòria intestinal prolongada s'associa a alteracions metabòliques com l'obesitat, la síndrome metabòlica i la resistència a la insulina. A més, diversos estudis han reportat que l'augment de la permeabilitat de l'epiteli intestinal pot facilitar l'entrada de potencials al·lèrgens alimentaris, augmentant així la sensibilització i els riscos d'al·lèrgia [201,202]. A més, la circulació sistèmica de LPS a causa d'una neutralització inadequada per part del sistema immunitari intestinal condueix a l'endotoxèmia metabòlica i inflamació sistèmica crònica de baix grau [203]. En aquest sentit, la disbiosi de la microbiota, la disfunció barrera i la inflamació de baix grau s'observen sovint en diversos estats de la malaltia, incloent la malaltia cardiovascular, el càncer, l'obesitat, les malalties respiratòries, la diabetis, la malaltia inflamatòria intestinal (IBD, de l'anglès), els trastorns cerebrals, la malaltia renal crònica i la malaltia hepàtica. Tot i que no s'ha establert el seu paper causal en el desenvolupament de la malaltia, s'han proposat tractaments per millorar l'estat de la malaltia restaurant la salut intestinal [88].

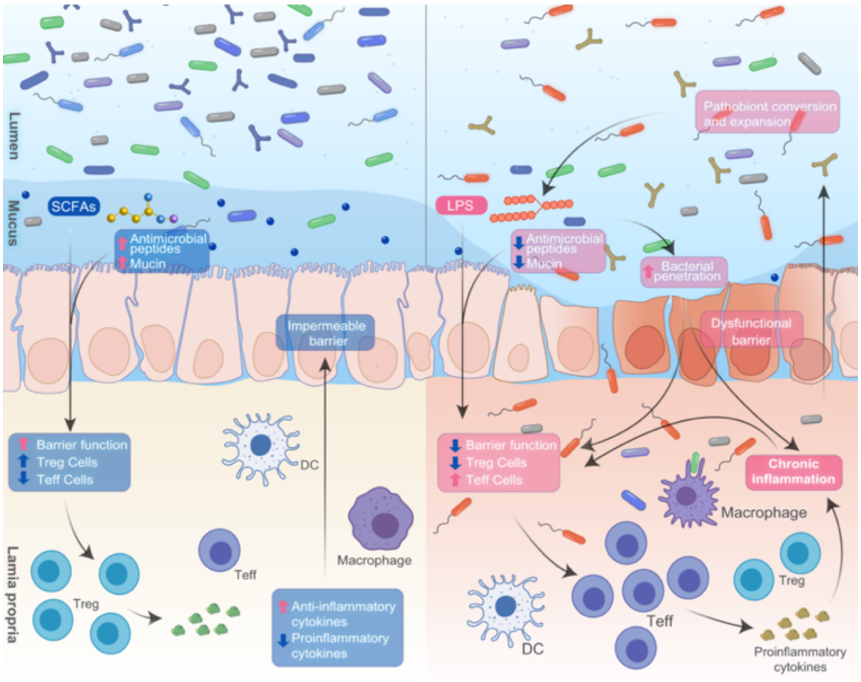

**Figura 3**. Barrera intestinal en estat sa i de malaltia. En un estat saludable, la barrera manté l'equilibri entre la microbiota, les capes de mucus i els àcids grassos de cadena curta (SCFAs), que promou una capa epitelial robusta i l'activació de cèl·lules Treg que secreten citocines antiinflamatòries. En estat de malaltia, la disrupció de la barrera permet el pas de LPS i bacteris, que condueix a l'activació de cèl·lules T efectores (per exemple, Th1, Th2, Th17), i l'augment de citocines proinflamatòries, desencadenant la inflamació crònica i l'exacerbació de la disfunció de barrera. Adaptat de Hou *et al.* [204].

### Models d'inflamació i barrera alterada: dieta de cafeteria i injecció de LPS

S'ha descrit àmpliament que una dieta equilibrada és essencial per mantenir un microbioma intestinal saludable, la integritat de la barrera intestinal i la tolerància immunitària. En canvi, una dieta desequilibrada, com la dieta occidental, pot conduir a una disminució de la diversitat de microbiota, disbiosi, alteració de la funció barrera i inflamació crònica [150]. Al laboratori, la dieta de cafeteria (CAF) s'utilitza per imitar la dieta occidental humana en experiments amb animals, ja que consisteix en aliments altament saborosos i densos en energia com salsitxes i magdalenes [205]. En comparació amb altres dietes altes en calories, la dieta CAF s'ha convertit en l'opció preferida per estudiar l'obesitat induïda per la dieta i la síndrome metabòlica en models animals [206]. Les rates alimentades amb aquesta dieta durant cinc setmanes van mostrar un major augment de pes en comparació amb els controls, i altres canvis van començar a augmentar [207].

Aquesta dieta es caracteritza per alts nivells d'hidrats de carboni i greixos simples, associats a la disbiosi, i baixos nivells de fibra, vitamines i minerals, nutrients que intervenen en el correcte funcionament de la barrera. En aquest sentit, la permeabilitat intestinal observada en l'obesitat s'ha relacionat amb disbiosi microbiana, patrons dietètics (contingut en sucre i greixos) i deficiències nutricionals [200]. D'una banda, la menor ingesta de fibra dietètica condueix a una disminució de la diversitat microbiana [208], mentre que un consum elevat de sucres i greixos simples condueix a una disminució de bacteris beneficiosos com *Lactobacillus* i *Bifidobacterium* i un augment de bacteris gram negatius rics en LPS [209]. D'altra banda, aquestes dietes s'associen amb criptes intestinals malmeses, reducció del nombre de cèl·lules caliciformes i canvis en la producció de mucines. A més, a causa del seu baix contingut en fibra, la microbiota catabolitza proteïnes endògenes i glicoproteïnes del mucus, donant lloc a un augment dels nivells de metabòlits nocius i a la degradació de la mucositat [210]. Alhora, a nivell de la capa epitelial, el consum excessiu crònic d'aquest tipus de dieta promou la pèrdua de proteïnes TJ [211]. Finalment, les dades d'estudis en animals suggereixen que el greix de la dieta és el principal macronutrient responsable de l'endotoxèmia postprandial i que tant la quantitat com la qualitat del greix de la dieta tenen efectes diferents sobre l'endotoxèmia metabòlica [212]. En aquest sentit, els persistents nivells circulants elevats de citocines inflamatòries associades a l'obesitat induïda, també poden alterar l'estructura i localització de les TJ [213].

Aquesta reducció de la mucositat i l'alteració de la permeabilitat condueix a una translocació del contingut luminal a la *lamina propria*, i augmenta la resposta immunitària de l'hoste al microbioma en permetre l'accés bacterià sense restriccions a l'epiteli [12], resultant en un augment de les endotoxines bacterianes en la circulació sistèmica i la inflamació intestinal i general [150]. La disrupció de l'homeòstasi intestinal afecta les cèl·lules de Tregs, resultant en la pèrdua de tolerància immune i respostes efectores aberrants, amb un augment de la secreció de citocines proinflamatòries a través de les vies Th1 i Th17 [214]. En conseqüència, els monòcits sanguinis són reclutats als teixits inflamats on alliberen una varietat de mediadors proinflamatoris, com ara TNF-α, factor infiltrant de macròfags, IL-1β, IL-6, IL-12 i IL-18 [156]. A més, l'activació persistent de neutròfils, l'augment d'infiltració i les baixes taxes d'apoptosi són freqüents en aquesta situació, contribuint a la cronificació de la inflamació intestinal i sistèmica [158].

Així, la dieta occidental o de cafeteria provoca danys intestinals amb posterior inflamació sistèmica a causa de l'entrada d'endotoxines derivades de la microbiota al torrent sanguini, donant lloc a malalties com l'obesitat o la síndrome metabòlica [215]. Aquesta situació també s'aborda al laboratori amb tractaments amb LPS o sulfat sòdic dextrà (DSS) en models animals, teixits intestinals humans *ex vivo* i estudis de cultius cel·lulars [216–219]. La injecció intraperitoneal d'LPS indueix una resposta inflamatòria sistemàtica i local que afecta la permeabilitat intestinal i agreuja la inflamació [218,219]. La inflamació crònica de baix grau associada a l'endotoxèmia metabòlica és un factor important en el desenvolupament de moltes malalties cròniques en animals i humans [212,220].

### Barrera intestinal i al·lèrgia alimentària

La funció de barrera immunitària intestinal és crucial per defensar-se dels al·lèrgens, i les interrupcions d'aquesta barrera poden contribuir al desenvolupament o exacerbació de les al·lèrgies alimentàries.

Normalment, la tolerància als antígens alimentaris es desenvolupa en edats primerenques de la vida, recolzada per factors com la lactància materna, que transfereix mediadors immunes de mare a fill. Aquest procés implica la participació de les DCs i la diferenciació de cèl·lules T ingènues en cèl·lules Treg, així com la producció d'IL-10, i sIgA o sIgG, que inhibeixen la sensibilització a al·lèrgens alimentaris específics i promouen la tolerància immunitària [221]. No obstant això, la interrupció de la tolerància fisiològica condueix a la producció de citocines proinflamatòries com IL-4, IL-5 i IL-13 per les cèl·lules epitelials intestinals i les DC, canviant la diferenciació dels limfòcits ingenus cap a respostes al·lèrgiques (a través de Th2) en lloc de tolerància [72].

Aquesta resposta al·lèrgica consisteix en l'expansió de mastòcits, basòfils i, en conseqüència, eosinòfils a la mucosa, acompanyada del desenvolupament de limfòcits B productors d'IgE. Els mastòcits, cèl·lules clau en el desenvolupament de l'al·lèrgia, expressen en la seva superfície el Fc$\varepsilon$RI, un receptor IgE d'alta afinitat, al qual s'uneix la IgE específica a l'al·lergogen produïda durant la fase de sensibilització. L'exposició posterior al mateix al·lergogen alimentari s'uneix amb l'IgE específica de l'al·lergen en mastòcits, desencadenant una resposta de fase efectora (desgranulació) caracteritzada per l'alliberament de mediadors químics, com histamina, prostaglandines i leucotriens, que indueixen símptomes al·lèrgics [222,223]. Per tant, l'augment de la producció total d'IgE està fortament associat amb la resposta al·lèrgica, fent que els nivells plasmàtics d'IgE siguin un marcador fiable quan se sospita al·lèrgia alimentària mediada per IgE [224].

La investigació sobre l'origen de la malaltia destaca diversos factors que predisposen, inicien o exacerben la immunitat alterada en malalties al·lèrgiques, incloent la disfunció inherent de la barrera epitelial, la pèrdua de tolerància immune, les alteracions en l'intestí i els microbiomes específics d'òrgans, la dieta i l'edat [202]. Per tant, la funció barrera intestinal té un paper fonamental en la prevenció o facilitació del desenvolupament de l'al·lèrgia alimentària. Una barrera alterada, un augment de la permeabilitat, la disbiosi o la desregulació del sistema immunitari de les mucoses poden contribuir al desenvolupament o exacerbació de les al·lèrgies alimentàries [225]. En primer lloc, un intestí permeable pot permetre que nivells més alts d'al·lèrgens travessin l'epiteli i arribin a les cèl·lules immunitàries, especialment als mastòcits, estimulant les reaccions al·lèrgiques [226]. En segon lloc, una microbiota sana promou la tolerància immunitària a proteïnes alimentàries inofensives promovent cèl·lules Treg, però el desequilibri de la microbiota pot sobre activar la resposta immunitària a aquests antígens [227]. A més, la desregulació de la resposta immunitària pot donar lloc a reaccions aberrants a les proteïnes de la dieta. Així mateix, aquesta disfunció de barrera epitelial i la cronicitat de l'estat inflamatori, podrien reduir el llindar de sensibilització a substàncies innòcues a causa de l'entorn inflamatori, precipitant potencialment la sensibilització al·lèrgica [228]. Una vegada més, també hi ha evidència que els mediadors alliberats dels mastòcits i altres citocines Th2 contribueixen a l'augment de la permeabilitat paracel·lular, sent un cercle viciós [229].

Les malalties relacionades amb la inflamació intestinal es caracteritzen per un augment de mastòcits i els seus productes de desgranulació a l'intestí prim i al còlon [230]. Els tractaments amb LPS també augmenten la resposta Th2 amb l'augment de la secreció d'IgE, el reclutament i desgranulació de mastòcits i l'activació d'eosinòfils [218,231].

En aquest sentit, la desregulació o alteració de la barrera intestinal podria produir inflamació intestinal, així com agreujar altres patologies relacionades amb la inflamació a nivell sistèmic.

### 3.2.3. Interacció dietètica amb la barrera intestinal

Com s'ha esmentat anteriorment, la dieta, reconeguda des de fa temps com un dels factors externs més importants que influeixen en la salut, té un impacte primordial en la salut intestinal. Els components dietètics interactuen directament amb el tracte gastrointestinal i, per tant, influeixen en la seva integritat i composició [232]. Mentre que s'ha demostrat que la dieta típica occidental resulta en un intestí permeable i inflamació crònica; Una dieta nutricionalment equilibrada, rica en fruites i verdures, és essencial per mantenir un microbioma intestinal saludable, preservar la integritat de la barrera intestinal, la tolerància immunitària i mantenir la fisiologia intestinal normal. A més, el consum de fruites i verdures en general, però també de proteïnes i pèptids vegetals, polifenols, àcids grassos $\omega$-3 i fibra dietètica s'han associat amb beneficis per a la salut intestinal [233]. En conseqüència, les nostres eleccions dietètiques influeixen en l'estructura, composició i funció de la microbiota, que interactuen amb l'epiteli intestinal i el sistema immunitari de les mucoses i per mantenir l'homeòstasi intestinal en un estat saludable [150].

En el context de la disbiosi, algunes teràpies basades en la microbiota, incloent prebiòtics i probiòtics, han sorgit com a tractaments prometedors [234]. Els probiòtics, com *Lactobacillus* i *Bifidobacterium*, són microorganismes vius que tenen un o més efectes beneficiosos sobre l'hoste quan es consumeixen en quantitats suficients, mentre que els prebiòtics són compostos que són indigestos pel GIT humà i es descomponen pel microbioma o microorganismes probiòtics [235]. Les principals fonts de prebiòtics inclouen fruites, verdures, llegums i cereals integrals, que contenen fibra dietètica i altres carbohidrats fermentables, també coneguts com carbohidrats accessibles per la microbiota (MAC) [233]. S'ha descrit que la disponibilitat de fibra insoluble i MACs podria influir en la composició de la microbiota. A més, el seu consum s'ha associat amb una millora de la permeabilitat paracel·lular mitjançant la regulació de l'expressió de la proteïna TJ [236]. Finalment, s'ha demostrat que el consum de MACs millora les situacions inflamatòries de baix grau induïdes per una dieta alta en greixos [183]. D'altra banda, una fibra dietètica insoluble com la quitina d'insectes s'ha relacionat àmpliament amb els beneficis actuals per a la salut, promovent el creixement de bacteris beneficiosos i inhibint el creixement d'alguns bacteris potencialment patògens. A més, també s'ha demostrat que els derivats de quitina tenen propietats antiinflamatòries [237].

Diversos estudis han demostrat que els pèptids bioactius derivats de proteïnes dietètiques poden influir en la salut intestinal modulant la funció barrera. En aquest sentit, s'ha descrit estimulen la producció i secreció de mucus, augmenten la secreció d'IgA i indueixen la proliferació cel·lular epitelial [238]. A més, els pèptids que poden afectar diferents fonts proteiques s'han relacionat amb l'augment de la diversitat de la microbiota i la regulació de la resposta immunitària [239]. Altres autors també assenyalen que el consum de glutamina podria millorar la salut intestinal participant en la renovació de les cèl·lules epitelials i augmentant l'expressió dels TJ [240]. No obstant això, es necessita més investigació sobre l'eficàcia de pèptids i aminoàcids bioactius de diverses i noves fonts proteiques, com els insectes, en el tractament o prevenció de malalties.

Altres compostos bioactius com els polifenols, que es troben principalment en fonts vegetals, exerceixen diversos efectes beneficiosos sobre la salut intestinal i la funció immune. S'ha suggerit que milloren la integritat intestinal modulant l'organització dels TJ i augmentant la seva expressió, millorant els defectes de barrera intestinal i l'endotoxèmia metabòlica induïda per una dieta alta en greixos [241,242]. De la mateixa manera, s'ha descrit que els àcids grassos $\omega$-3 tenen aquestes propietats, i també que estan implicats en la regulació de les vies de senyalització per reduir la inflamació epitelial, atenuant la producció de mediadors proinflamatoris i regulant la producció de mediadors pro-resolutius [243].

En conclusió, la dieta és un factor clau que influeix en la salut intestinal, ja que se sap que certs aliments i nutrients potencien la funció barrera intestinal [236]. Més investigacions sobre aquests components dietètics, així com els aliments que poden contenir-los, podrien proporcionar informació valuosa per promoure la salut intestinal i prevenir malalties intestinals. A més, amb totes les evidències dels apartats anteriors, podríem concloure que la barrera intestinal és una interfície dinàmica que regula l'entrada d'aliments i patògens al tracte gastrointestinal, afectant el manteniment general de la salut.

Finalment, pel que fa al reconeixement d'aliments, els components dietètics que arriben a l'intestí interaccionen amb els receptors del gust, que no es limiten a la cavitat oral sinó que també estan presents en el tracte intestinal [6]. Com es descriu en la secció següent, aquests receptors intervenen en vies de senyalització associades amb funcions fisiològiques més enllà del seu paper en el gust i s'ha suggerit que influeixen en diverses respostes metabòliques i immunitàries [244]. No obstant això, l'efecte de diferents patrons dietètics, com ara noves fonts proteiques, sobre els receptors intestinals del gust i els conseqüents efectes sobre la barrera intestinal, però també sobre la salut general, necessita ser investigat més a fons i s'aborda en la present tesi.

### 3.3. Receptors del gust

El consum de components alimentaris activa una cascada de respostes dins del nostre cos, influint en l'homeòstasi del nostre cos. Tradicionalment, el reconeixement dels components dels aliments ha estat duta a terme pels receptors del gust, proteïnes especialitzades situades predominantment en papil·les gustatives disperses per tota la cavitat oral [245]. Aquests receptors juguen un paper fonamental en el reconeixement de compostos exògens presents en aliments i begudes, així com altres substàncies ingerides. Els senyals que generen es transmeten a través de nervis gustatius aferents a les estructures cerebrals encarregades del processament central del gust, donant forma a la nostra experiència sensorial del sabor [246]. No obstant això, la influència dels receptors del gust s'estén molt més enllà dels confins de la cavitat oral. Se'n coneix la seva presència en teixits i òrgans extraorals, que abasten des del cervell i la pell fins al sistema reproductor i el tracte gastrointestinal [247]. L'abundància i la distribució en diferents teixits dels receptors del gust planteja la possibilitat de la seva implicació en altres funcions fisiològiques [248].

Els receptors del gust són sensibles a diferents gustos, inclosos el dolç, l'amarg, el salat, l'àcid i l'umami. Els gustos salat i àcid són detectats per canals iònics; mentre que el canal epitelial de sodi (ENaC) respon als estímuls de sodi, el canal iònic selectiu de protons Otop1 i la disminució del pH són encarregats de la transducció dels senyals àcids [249,250]. En canvi, els receptors umami, dolços i amargs pertanyen a la superfamília dels receptors acoblats a proteïnes G (GPCR) i es divideixen en dos tipus. Els noms estàndard segueixen la descripció de "receptor del gust, tipus X, membre X", abreujat en majúscules / majúscules (TASXRX) per als humans, i amb minúscules per als rosegadors (TasXrX) [251,252]. Els TAS1R, responsables del sabor dolç i umami, són GPCRs amb un llarg amino-terminal i funcionen en dímers utilitzant

TAS1R3 com a subunitat obligada. Concretament, l'heterodímer TAS1R2-TAS1R3 és l'encarregat de detectar la dolçor, mentre que els L-aminoàcids i ribonucleòtids interaccionen amb TAS1R1-TAS1R3 per formar la sensació gustativa coneguda com umami [253,254]. Els compostos amargs són detectats per TAS2R, un receptor també tipus GPCRs amb un residu curt, i poden actuar com a monòmers [255]. En els paràgrafs següents, TAS1R i TAS2R es descriuran amb més detall, ja que han estat un dels temes d'estudi d'aquesta tesi.

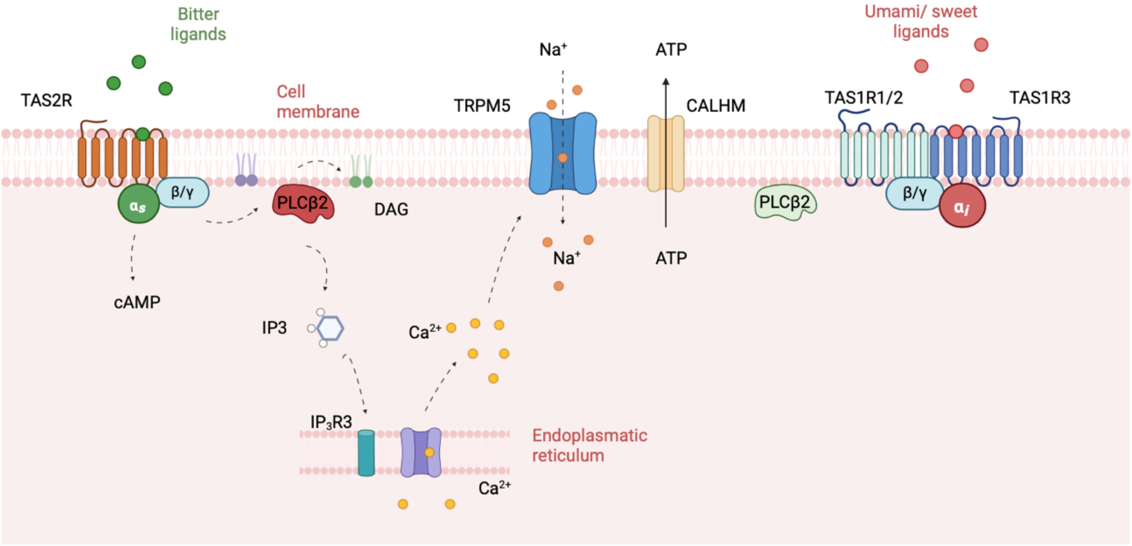

**Figura 4.** Via de senyalització del receptor del gust GPCR: quan el lligand s'uneix a TAS1R1/TAS1R3, TAS1R2/TAS1R3 o TAS2R, la proteïna G es dissocia i activa la producció de IP3, la secreció de $Ca^{2+}$ del reticle endoplasmàtic i el consegüent gradient de $Na^+$ cap a l'interior cel·lular. Això acaba amb la secreció d'ATP, entre altres molècules efectores que indueixen la percepció del gust o desencadena l'activació de diferents funcions. Creat amb BioRender.com.

Les vies gustatives a través dels receptors gustatius pertanyents a la família GPCRs, és a dir, el mecanisme de transducció dolç, umami i amarg, comencen amb la unió del lligand gustatiu al receptor específic, induint un canvi conformacional (figura 4). Llavors, les proteïnes G heterotrimèriques unides a GTP (subunitats α, β i γ) es dissocien i les subunitats β i γ- activen la fosfolipasa C2 unida a la membrana (PLCβ2), induint així la producció d'inositol-1,4,5-trifosfat (IP3) i diacilglicèrids (DAG) [248,256,257]. L'IP3 s'uneix al seu receptor situat a la membrana del reticle endoplasmàtic, activant així els canals de calci i provocant un augment dels ions citoplasmàtics de $Ca2^+$. Aquest calci activa el receptor transitori de potencial canal catiònic de la subfamília M, membre 5 (TRPM5) [258] que, juntament amb altres canals de $Na^+$, augmenta els ions $Na^+$. Les cèl·lules responen al senyal de sodi via despolarització i flux extracel·lular del neurotransmissor adenosina-5'-trifosfat (ATP) als nervis gustatius aferents, a través del modulador de l'homeòstasi càlcica 1 CALHM1 o CALHM1/CALHM3, i condueixen a la percepció del gust [259]. No obstant això, se sospita que aquesta no és l'única manera d'activar la via de senyalització, ja que la dissociació de la subunitat α-gustducina quan un lligand s'uneix a GCPR sembla elevar també els nivells intracel·lulars de $Ca2^+$ mitjançant la hidròlisi de l'AMPc, el que torna a donar lloc a l'exocitosi dels neurotransmissors[260,261]. En altres localitzacions, per exemple, a l'intestí, s'han descrit aquestes vies de senyalització per acabar amb l'alliberament d'hormones o altres mediadors de la funció paracrina [262].

### Receptors del gust dolços i umami (TAS1R per a humans, Tas1r per a rates)

TAS1R, que comprèn el receptor del gust tipus I membre 1 (TAS1R1), 2 (TAS1R2) i 3 (TAS1R3), funcionen com heterodímers, cadascun dels quals posseeix un gran domini extracel·lular fusionat als set dominis transmembrana [263].

TAS1R2/TAS1R3 interactua amb compostos de sabor dolç com la sacarosa, la fructosa, els edulcorants artificials i certs D-aminoàcids, provocant la sensació de sabor dolç [263]. S'ha descrit la localització extraoral d'aquest receptor que li confereix la seva implicació en diversos processos fisiològics; Per exemple, juga un paper en la secreció d'insulina, el creixement dels teixits i la regeneració dels músculs esquelètics, contribuint així al processament saludable de compostos dolços [264]. La seva participació en la secreció d'insulina pot ocórrer directament, a causa de la seva expressió en cèl·lules-$\beta$ pancreàtiques, o a través de la seva implicació en l'alliberament de GLP-1, a causa de la seva expressió en cèl·lules-L enteroendocrines [265,266].

D'altra banda, el terme "umami" prové de la paraula japonesa "umai" que significa "deliciós” [246], i s'associa amb aliments com la vedella, embotits, bolets, salsa de soja, formatge curat, marisc i tomàquet [267]. Així, l'agonista de TAS1R1/TAS1R3 incloïa L-aminoàcids, com glutamat, aspartat, alanina, serina, asparagina, arginina, histidina, treonina, glutamina i 5′-ribonucleòtids, (monofosfat d'inosina 5′; IMP), i també alguns pèptids, sent L-glutamat el principal estímul amb gust umami, que sovint es troba en la dieta com glutamat monosòdic (MSG) i la seva forma de sal [263].

Els receptors umami extraorals s'han relacionat amb la modulació de l'alliberament hormonal (per exemple, grelina i colecistoquinina), a causa de la seva ubicació estomacal i intestinal [268]. A més, algunes evidències suggereixen la seva implicació en la regulació inflamatòria i de la funció intestinal [253,269].

### Receptors del gust amarg (TAS2R per a humans, Tas2r per a rates)

La sensació de sabor amarg pot ser desencadenada per una àmplia gamma de compostos amb diverses estructures químiques, des de sals simples fins a molècules grans i complexes, moltes de les quals es consideren tòxiques [255]. Els humans expressem aproximadament 26 subtipus de receptors del gust amarg, mentre que les rates expressen al voltant de 37 subtipus diferents [251,270], cadascun amb la seva pròpia nomenclatura única, tal com van descriure prèviament Descamps-Solà *et al.* [6]. A més, cada subtipus és susceptible de ser activat per compostos amargs específics o un ampli espectre d'ells [271]. En aquest sentit, s'ha identificat una llarga llista d'agonistes específics per a alguns receptors amargs, incloent compostos alimentaris com el resveratrol, l'epicatequina galat, la quinina i edulcorants artificials com l'acesulfam K i la sacarina [272,273]. A més, certs TAS2R han demostrat sensibilitat a pèptids i aminoàcids específics [274–278].

Més enllà del seu paper en la percepció del gust, la seva presència al cor, bufeta, vies respiratòries i múscul llis gastrointestinal ha apuntat la seva participació en la relaxació muscular i la broncodilatació [268]. Addicionalment, TAS2R també es localitza en les cèl·lules epitelials intestinals i s'ha demostrat la seva participació en la regulació de la ingesta d'aliments, disminuint-la a través de canvis en la secreció hormonal [264,279]. A més, aquests receptors també s'han associat a mecanismes de defensa intestinal i resposta immunitària [280].

### 3.3.1. Receptors intestinals del gust

S'ha descrit que els receptors del gust amarg, dolç i umami s'expressen a l'intestí i s'ha trobat que detecten una àmplia gamma de molècules endògenes i nutrients dietètics que arriben al lumen intestinal. En aquest sentit, la composició del contingut luminal intestinal varia considerablement amb la dieta, necessitant que l'epiteli intestinal detecti i respongui a aquests canvis significatius per regular les seves funcions en conseqüència [281]. Es considera que aquests receptors del gust són activats per certs nutrients i juguen un paper important en diverses activitats fisiològiques intestinals, a través de xarxes quimiosensorials similars als receptors linguals [262].

Així, a l'intestí prim, es localitzen principalment a les cèl·lules enteroendocrines. Després de l'activació per lligands específics, s'ha demostrat que els receptors del gust tant de tipus 1 com de tipus 2 desencadenen l'alliberament de diverses hormones i neurotransmissors, com el pèptid similar al glucagó 1 (GLP-1)[282], la colecistoquinina (CCK) [283] i la grelina [284,285]. Aquestes molècules modulen l'absorció i el metabolisme dels nutrients, la sacietat, la ingesta d'aliments, el comportament alimentari i la motilitat intestinal, mantenint així l'homeòstasi en resposta als components de la dieta [286–288]. Aquesta evidència reforça la importància de la funció intestinal en la salut general més enllà de l'intestí. Per exemple, un mal funcionament dels receptors intestinals del gust pot provocar trastorns digestius i desencadenar obesitat, diabetis tipus 2 i malalties gastrointestinals [245,253,289]. D'altra banda, l'alteració genètica en TAS2R38 s'ha relacionat amb la disbiosi intestinal, l'alteració de la ingesta i la mala regulació de la immunitat innata, que podrien conduir a altres alteracions com la malaltia de Parkinson [290].

D'altra banda, TAS1R i TAS2R també s'han trobat en diversos tipus cel·lulars de l'epiteli intestinal, com enteròcits, Tuft, Paneth, caliciformes i cèl·lules M, per tant, els receptors del gust intestinal estan implicats en la regulació de la funció barrera intestinal i les respostes immunitàries [256,291]. Les cèl·lules Tuft intestinal s'han associat recentment amb la participació en l'inici de la immunitat tipus 2 a través de l'alliberament d'IL-25, després de l'activació del receptor intestinal del gust amarg per paràsits o microbis [292]. A més, Howitt *et al.* va apuntar que Tas1r3 té un paper important en la regulació de l'homeòstasi de les cèl·lules Tuft a l'intestí prim, modulant així la sensibilitat als estímuls luminals durant l'expansió de la resposta immunitària de tipus 2 [293]. D'altra banda, la presència de TAS2R en les cèl·lules de Paneth i caliciformes va suggerir la seva participació en la producció de pèptids antimicrobians i mucus [280,294].

Alguns estudis han relacionat l'alteració de TASR o les molècules de la seva cascada de senyalització, amb el deteriorament intestinal i la resposta inflamatòria sistèmica. Per exemple, la colitis induïda per DSS en ratolins knockout de α-gustducina donava lloc a símptomes de malaltia inflamatòria intestinal agreujats, a l’augment de la infiltració de cèl·lules immunitàries i l’alliberament de mediadors inflamatoris respecte dels ratolins control [295]. Per contra, un altre estudi sobre l'activació de TAS2Rs i la resposta metabòlica, va observar una reducció de les citocines proinflamatòries que també suggeria la regulació de les respostes antiinflamatòries a través d'aquests receptors [287]. A més, l'expressió de TAS2Rs en macròfags, monòcits, mastòcits, neutròfils i en limfòcits B i T, ens porta a pensar en el seu paper en la immunitat innata i adaptativa [256]. Per tant, l'activació d'aquests receptors modula la funció immunitària epitelial, la secreció de moc i la producció de pèptids antimicrobians, influint en la composició de la microbiota intestinal i l'homeòstasi immune, localment i més enllà del tracte gastrointestinal [296].

### 3.3.2. Receptors del gust i funció immunitària

A més del tracte gastrointestinal, els receptors del gust han estat àmpliament implicats en la resposta immune en altres localitzacions. Els TAS2R s'han trobat recentment en neutròfils de sang perifèrica, monòcits, i també en limfòcits en repòs i activats, i s'ha descrit que contribueix a la resposta adaptativa [297]. De la mateixa manera, s'ha descrit que els macròfags pulmonars humans expressen receptors del gust amarg i el tractament amb agonistes amargs suprimeixen la producció de citocines [298]. Tant TAS1R com TAS2R s'han trobat en leucòcits circulants [299]. Els TAS2R han estat implicats en la modulació antiinflamatòria mitjançant la regulació de l'alliberament d'IL-6, en fibroblasts gingivals humans [273]. De la mateixa manera, la TAS2R gingival respon a senyals bacterians que indueixen respostes immunitàries innates de l'hoste, com la secreció d'AMPs, per evitar el creixement excessiu de bacteris orals i regular la composició microbiana oral [300]. Hollenhorst *et al.* va suggerir que la detecció del gust amarg en les cèl·lules del raspall traqueal desencadena la resposta immunitària innata a la infecció bacteriana [301]. A més, un estudi en cèl·lules de raspall uretral va demostrar que l'activació dels receptors amarg i umami condueix a l'alliberament d'acetilcolina i, en conseqüència, a l'eliminació de patògens del tracte urinari [302].

D'altra banda, la inflamació aguda, però també una inflamació crònica de baix grau associada a patologies com l'obesitat, sembla alterar el sistema gustatiu. A més, el paper immunitari dels receptors del gust posa de manifest més vincles entre la immunitat i el gust [303]. En obesitat, hi ha evidències considerables que suggereixen que la percepció del gust està alterada i que TAS1R, TAS2R o les molècules associades a la seva senyalització i transducció estan implicades en la regulació de l'estat inflamatori, alhora que actuen en la retroalimentació negativa per regular la ingesta de nutrients i mantenir l'homeòstasi energètica [304].

### 3.3.3. Modulació de l'expressió gènica i proteica dels receptors del gust

A més de l'activació o inhibició, s'ha descrit que l'expressió dels receptors intestinals del gust pot ser modulada per la dieta i els canvis en situacions patològiques, amb conseqüències evidents per a la seva resposta fisiològica [305]. A nivell oral, l'exposició crònica a sacarosa inhibeix les respostes gustatives a concentracions més altes d'estímuls dolços, el que suggereix que un receptor pot ser sensibilitzat i desregulat després del consum crònic d'un agonista [306]. De la mateixa manera, la sobreexposició a llarg termini a gustos apetitius, com el glutamat monosòdic o la sacarina, condueix a una desregulació dels receptors específics (TAS1R1 o TAS1R2, respectivament) en les papil·les gustatives dels animals, augmentant la possibilitat que la modulació dirigida als receptors del gust pugui oferir estratègies potencials per combatre l'obesitat [307]. D'altra banda, Lin *et al.* van mostrar un augment en l'expressió gènica de Tas2r induït per la inflamació en les llengües de ratolins, resultant en una alteració de la percepció del gust amarg i la possibilitat de més infeccions [308]. No obstant això, aquesta modulació de l'expressió del receptor també s'ha observat en teixits extraorals, i alguns autors han investigat la modulació a nivell intestinal. Per exemple, Zhang *et al.* va informar d'un augment de l'expressió d'ARNm intestinal de Tas1r2 i Tas1r3 quan les rates consumien sacarosa i fructosa amb o sense dieta alta en greixos [309]. Així mateix, Wang *et al.* va descriure que els humans obesos i els models animals tenen alterada l'expressió intestinal de TAS1R i TAS2R, i, conseqüentment, alterada la senyalització quimiosensorial, com la secreció de grelina [310]. A més, en un model de ratolí de diabetis tipus 2 induïda per una dieta alta en greixos i estreptozotocina, es va veure augmentada l'expressió de Tas1r3 i TRPM5 del còlon, mentre que una dieta amb quinoa com a tractament corrector va revertir aquests canvis [311].

Com s'ha descrit anteriorment, la microbiota té un gran impacte en la funció intestinal. Així, s'ha proposat un concepte relativament nou en què la microbiota intestinal té un paper en la inflamació mediada pels receptors del gust. Els canvis en la microbiota podrien afectar l'expressió de les GPCRs del gust i la seva cascada de senyalització, alterant així la immunitat innata i el metabolisme de l'hoste [304]. Swartz *et al.* va demostrar una sobre expressió del gen de la proteïna intestinal corresponent a TAS1R3 en ratolins lliures de gèrmens, associat amb una major preferència per les solucions de sacarosa [312]. A més, s'ha descrit que els SCFAs derivats de la fermentació de la microbiota influeixen en l'expressió de TAS1R [313]. De la mateixa manera, un altre estudi recent ha demostrat una regulació ascendent de Tas2r depenent de la microbiota en ratolins sotmesos a una dieta alta en greixos a llarg termini [314], mentre que Latorre *et al.* va observar que el tractament amb antibiòtics pot revertir la desregulació T2R138 induïda per la dieta [305]. Com que és ben sabut que les dietes alteren la composició de la microbiota [315], és raonable sospitar que això podria influir en l'expressió dels receptors del gust.

Totes aquestes investigacions suggereixen que l'expressió dels receptors del gust a l'intestí pot veure's afectada per compostos dietètics específics i patologies, però l'evidència segueix sent limitada. Es necessiten més estudis per explorar com diferents dietes o compostos dietètics modulen l'expressió dels receptors del gust i la potencial afectació de la funció fisiològica. Donada la importància d'un intestí sa en la prevenció de la inflamació crònica, la disfunció metabòlica i les malalties inflamatòries, i el vincle intrigant entre la funció dels receptors del gust intestinal, la integritat de la barrera intestinal i els resultats sistèmics de salut, la investigació en aquesta àrea és fonamental. Comprendre la relació entre els components de la dieta, la funció intestinal, l'expressió dels receptors del gust i les respostes sistèmiques és essencial per desenvolupar estratègies dietètiques específiques que promoguin la salut i el benestar generals.

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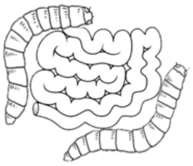
# HYPOTHESIS AND OBJECTIVES



# HYPOTHESIS AND OBJECTIVES

Given the rapid growth of the world's population and increasing environmental concerns, the search for sustainable protein sources has become imperative. Insects have emerged as a high-quality alternative protein source, comparable to other conventional sources, rich in bioactive compounds, and considered to be environmentally friendly to produce. However, their bioactivity for human health has been poorly analysed and further studies are needed.

The gastrointestinal tract (GIT) is the largest interface in the body exposed to the external environment. It plays a crucial role in food composition sensing, processing and absorption, as well as in the host's immune defence. Disruption of the intestinal barrier can allow endotoxins to enter the bloodstream, triggering systemic inflammation and metabolic disorders. Therefore, maintaining gut health is essential for overall well-being. In this sense, it is well known that dietary components have a significant impact on intestinal health and barrier function. For example, diets high in saturated fat and sugar have been implicated in gut dysbiosis and inflammation, while fibre-rich foods and other natural bioactive compounds are known to promote a healthy gut function.

With regard to food recognition, taste receptors have been identified as potential targets that are also influenced by food components. Traditionally associated with the oral cavity, these receptors are now known to be expressed in several extra-oral sites, such as the GIT, where they appear to regulate some important functions. However, there is still a lack of evidence regarding the modulation of taste receptors by dietary protein and its impact on gut and general health.

For all of the above, **we hypothesise that insect consumption, through its interaction with the gastrointestinal tract and, specifically, through intestinal taste receptors, could potentially modulate intestinal barrier function and thereby exert effects on overall well-being in health and disease.**

To demonstrate our hypothesis, we set the following specific objectives:

### Objective 1. To investigate the effects of low-dose chronic insect consumption on intestinal function and overall health, in both healthy and inflammatory conditions

Insects are rich in protein and provide a variety of other nutrients, including mono- and polyunsaturated fatty acids, vitamins and minerals. Although there is some evidence to suggest potential benefits of insect consumption, research into the effects of insects on gut health, particularly in relation to inflammation and permeability, remains limited. Therefore, in order to address this research gap, we first aimed to determine the acute *ex vivo* effects of insects on the intestinal immune response in human colon samples, compared to more conventional protein sources such as beef and almond. In addition, the effects of their chronic consumption *in vivo* in a disturbed intestinal pathology have not been extensively studied. In this sense, we aimed to study the effects of insect consumption on intestinal and general health, in terms of metabolism, inflammation, allergenicity and tissue morphology, in a healthy and LPS-induced intestinal dysfunction rats.

**Objective 2. To study the modulation of intestinal taste receptor gene expression by insect consumption and its implications in different health conditions**

Extra-oral taste receptors, as intestinal taste receptors, have been postulated as key targets in triggering metabolic and inflammatory responses. Their expression has been suggested to be modulated by dietary components or pathological status, but the effect the effect of insect supplementation on taste receptor expression has not been investigated. In this sense, our aim was to determine the effect of chronic low-dose insect supplementation on the intestinal taste receptor expression and to know its health implications, both at the intestinal and systemic level, in healthy and LPS-induced intestinal dysfunction rats.

**Objective 3. To analyse the effect of the chronic consumption of insects as unique source of protein on intestinal health and overall well-being in both healthy and diet-induced obesogenic rats**

Due to its content of amino acids and bioactive peptides, insect consumption has been postulated to induce different intestinal, inflammatory, and metabolic responses compared to the consumption of a conventional protein source. Although some studies have demonstrated acute effects of insect consumption or partial substitution of conventional protein sources, there is a lack of evidence on the health effects of chronic consumption of insect-based diets as a single protein source. Therefore, we aimed to evaluate the broad effects of insect-based diets on various aspects of health and physiology and compare them with those induced by conventional protein sources in both healthy and diet-induced obesogenic models.

# HIPÒTESI I OBJECTIUS

Donat el ràpid creixement de la població mundial i les creixents preocupacions ambientals, la recerca de fonts de proteïnes sostenibles és urgent i necessària. Els insectes han sorgit com una font de proteïna alternativa d'alta qualitat, comparable a altres fonts convencionals, rica en compostos bioactius i la seva producció es considera respectuosa amb el medi ambient. No obstant això, la seva bioactivitat sobre la salut humana ha estat poc analitzada i calen més estudis.

El tracte gastrointestinal (GIT) és la interfície més gran del cos exposada al medi extern. Té un paper crucial en la detecció, processament i absorció dels components dels aliments, així com en la defensa immunitària de l'hoste. La disrupció de la barrera intestinal pot permetre que les endotoxines entrin al torrent sanguini, desencadenant inflamació sistèmica i trastorns metabòlics. Per tant, mantenir la salut intestinal és essencial per al benestar general. En aquest sentit, és ben sabut que els components dietètics tenen un impacte significatiu en la salut intestinal i en la funció barrera. Per exemple, les dietes riques en greixos saturats i sucres han estat implicades en la disbiosi intestinal i la inflamació, mentre que els aliments rics en fibra i altres compostos bioactius naturals són coneguts per promoure una funció intestinal saludable.

Pel que fa al reconeixement d'aliments, els receptors del gust s'han identificat com a dianes potencials que, alhora, són modulades pels components dels aliments. Tradicionalment associats a la cavitat oral, actualment se sap que aquests receptors s'expressen en diversos llocs extra-orals, com el GIT, on semblen regular algunes funcions importants. No obstant això, encara hi ha una manca d'evidència sobre la modulació dels receptors del gust per part de proteïnes de la dieta, i l'impacte que això pot tenir en l'intestí i la salut general.

Per tot l'anterior, **plantegem la hipòtesi que el consum d'insectes, a través de la seva interacció amb el tracte gastrointestinal i, específicament, a través dels receptors intestinals del gust, podria modular potencialment la funció de barrera intestinal i, per tant, exercir efectes sobre el benestar general, en estat de salut i malaltia.**

Per demostrar la nostra hipòtesi, vam establir els següents objectius específics:

**Objectiu 1. Investigar els efectes del consum crònic d'insectes a dosis baixes sobre la funció intestinal i la salut en general, tant en condicions saludables com inflamatòries**

Els insectes són rics en proteïnes i proporcionen una gran varietat d'altres nutrients, incloent-hi àcids grassos mono i poliinsaturats, vitamines i minerals. Tot i que hi ha algunes evidències que suggereixen potencials beneficis del consum d'insectes, la investigació dels efectes del consum d'insectes sobre la salut intestinal, particularment en relació amb la inflamació i la permeabilitat, segueix sent limitada. Per tant, per abordar aquest buit en la recerca, en primer lloc ens vam proposar determinar els efectes aguts *ex vivo* dels insectes sobre la resposta immunitària intestinal en mostres de còlon humà, en comparació amb fonts de proteïnes més convencionals com la carn de boví i l'ametlla. A més, els efectes del seu consum crònic *in vivo* en situació intestinal alterada, no han estat àmpliament estudiats. En aquest sentit, el nostre objectiu era estudiar els efectes del consum d'insectes sobre la salut intestinal i general, en termes de metabolisme, inflamació, al·lergenicitat i morfologia tissular, en rates sanes i amb disfunció intestinal induïda per LPS.

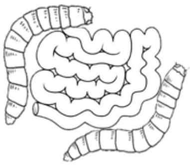
**Objectiu 2. Estudiar la modulació de l'expressió gènica dels receptors del gust intestinal pel consum d'insectes i les seves implicacions en diferents condicions de salut**

Els receptors del gust extra-orals, com són els receptors intestinals del gust, s'han postulat com a dianes clau per desencadenar respostes metabòliques i inflamatòries. S'ha suggerit que la seva expressió està modulada per components dietètics, per l'estat de salut o per l'edat, entre d'altres, però no s'ha investigat l'efecte de la suplementació d'insectes sobre l'expressió dels receptors del gust. En aquest sentit, el nostre objectiu va ser determinar l'efecte de la suplementació crònica d'insectes a dosis baixes sobre l'expressió dels receptors intestinals del gust i conèixer les seves implicacions per a la salut, tant a nivell intestinal com sistèmic, en rates sanes i amb disfunció intestinal induïda per LPS.

**Objectiu 3. Analitzar l'efecte del consum crònic d'insectes, com a font única de proteïnes, sobre la salut intestinal i el benestar general, tant en rates sanes com en situació d'obesitat induïda per la dieta**

Degut al seu contingut en aminoàcids i pèptids bioactius, s'ha postulat que el consum d'insectes pot induir diferents respostes intestinals, inflamatòries i metabòliques en comparació amb el consum d'una font proteica convencional. Encara que alguns estudis han demostrat efectes aguts del consum d'insectes o de la substitució parcial de les fonts proteiques convencionals, hi ha una manca d'evidència dels efectes sobre la salut del consum crònic de dietes basades en insectes com una única font proteica. Per això, el nostre objectiu va ser avaluar els amplis efectes de les dietes basades en insectes en diversos aspectes de la salut i la fisiologia, i comparar-los amb els induïts per fonts proteiques convencionals, tant en models saludables com en obesitat induïda per la dieta.

# RESULTS



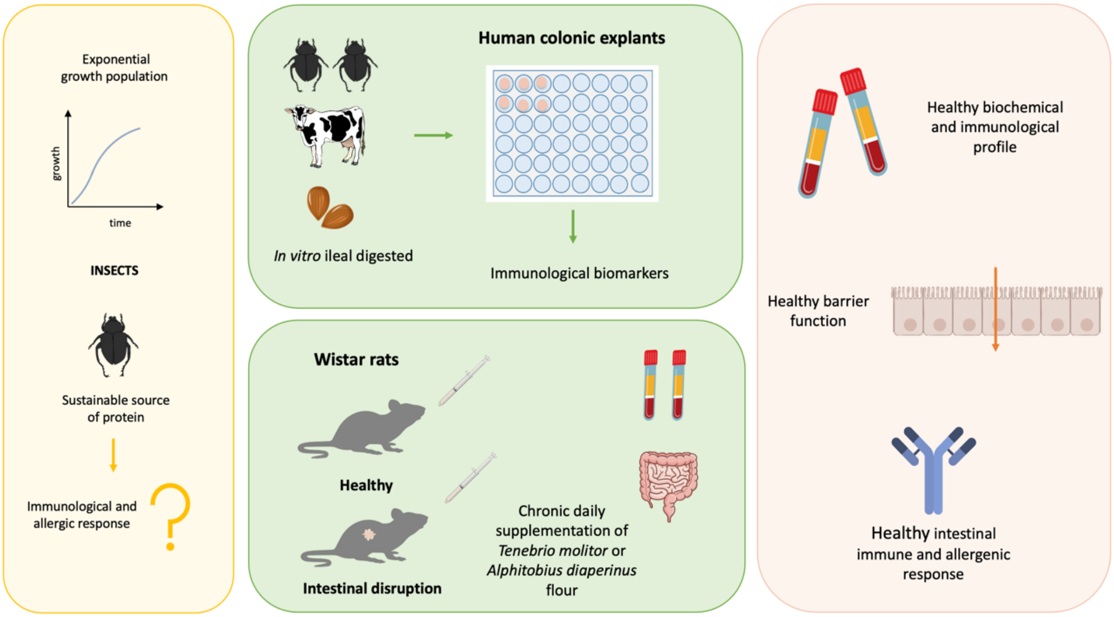
# PART 1

## Investigation of the effects of low-dose chronic insect consumption on intestinal function and overall health, in both healthy and inflammatory conditions

### Manuscript 1

Assessing the impact of insect protein sources on intestinal health and disease: insights from human *ex vivo* and rat *in vivo* models

*Published in Food and Function, 2 April 2024*



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Cite this: *Food Funct.*, 2024, **15**, 4552

# Assessing the impact of insect protein sources on intestinal health and disease: insights from human *ex vivo* and rat *in vivo* models

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Received 22nd January 2024,
Accepted 1st April 2024

DOI: 10.1039/d4fo00381k

rsc.li/food-function

The exploration of edible insects, specifically *Alphitobius diaperinus* and *Tenebrio molitor*, as sustainable sources of protein for human consumption is an emerging field. However, research into their effects on intestinal health, especially in relation to inflammation and permeability, remains limited. Using *ex vivo* and *in vivo* models of intestinal health and disease, in this study we assess the impact of the above insects on intestinal function by focusing on inflammation, barrier dysfunction and morphological changes. Initially, human intestinal explants were exposed to *in vitro*-digested extracts of these insects, almond and beef. Immune secretome analysis showed that the inflammatory response to insect-treated samples was comparatively lower than it was for samples exposed to almond and beef. Animal studies using yellow mealworm (*Tenebrio molitor*) and buffalo (*Alphitobius diaperinus*) flours were then used to evaluate their safety in healthy rats and LPS-induced intestinal dysfunction rats. Chronic administration of these insect-derived flours showed no adverse effects on behavior, metabolism, intestinal morphology or immune response (such as inflammation or allergy markers) in healthy Wistar rats. Notably, in rats subjected to proinflammatory LPS-induced intestinal dysfunction, *T. molitor* consumption did not exacerbate symptoms, nor did it increase allergic responses. These findings validate the safety of these edible insects under healthy conditions, demonstrate their innocuity in a model of intestinal dysfunction, and underscore their promise as sustainable and nutritionally valuable dietary protein sources.

## Introduction

There is an urgent need nowadays to feed an exponentially growing human population. As a result, the demand for protein is expected to increase by 70% in the next 30 years.[1] Current food research therefore focuses on incorporating foods derived from novel and sustainable protein sources such as insects.[2,3] Although insects have long been a common component of the human diet, in Western countries entomophagy is still in its early stages.[4] However, several reasons support incorporating insects into our diets as a valuable source of protein.

Firstly, insects contain a large amount of protein, accounting for over 50 percent of their crude weight. This abundant protein has high biological quality thanks to the presence of all essential amino acids.[5–7] Insects also contain large amounts of unsaturated fatty acids, minerals, and vitamins.[8] In addition, insects have been identified as a source of bioactive compounds with anti-hypertensive, antidiabetic, antioxidant, and anti-inflammatory activity.[9]

Also in support of insect consumption is the fact that insect production is more environmentally sustainable than the production of conventional livestock.[8] Indeed, insect-based protein is emerging as a cost-effective alternative to meat protein since its production employs minimal resources, requires less water and less land, and is responsible for a much lower carbon footprint than the production of meat protein.[4,10] Moreover, since insects can feed on bio-waste (such as mushroom waste and fruit peels), they can mitigate the environmental impact by turning waste into compost.[11]

For all these reasons, in the not-too-distant future insect protein will probably become a component of the total protein consumed by humans.[5] To enhance social acceptance, insect protein can also be processed into food ingredients such as flour, which can then be incorporated into food products.[12]

The yellow mealworm *Tenebrio molitor*, and the lesser mealworm *Alphitobius diaperinus* (Coleoptera: family Tenebrionidae),

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are both larvae of the beetles that grow in flour and grain cereals. These edible larvae possess significant nutritional value as they encompass a full spectrum of amino acids that are rich in essential fatty acids and vitamins as well as a higher mineral content (including calcium, copper, magnesium, iron and zinc) than conventional meats and eggs.[10,13–15] *Tenebrio molitor* commonly breeds in Europe and, together with *Alphitobius diaperinus*, is the main species used for farm-animal feed and considered for human consumption.[10]

Recent reports from the European Food Safety Authority (EFSA) positively evaluated both these mealworms as novel foods suitable for human consumption.[16,17] The safety profiles of *T. molitor* and *A. diaperinus* larvae as novel foods are based on nutritional studies that assessed the microbiological risk of zoonosis, heavy metal contamination and allergy. However, the EFSA recommends that further research should be conducted in this area.[14,17]

Recent studies have suggested beneficial effects of edible insects in terms of inflammation and intestinal health. For instance, a study with *Tenebrio molitor* larvae powder described its ability to attenuate pathologic changes in colon tissue and down-regulate the expression of inflammatory cytokine genes in mice (DSS)-induced colitis.[18] Moreover, an *in silico* analysis indicated that *Tenebrio molitor* could be a source of peptides with anti-inflammatory activity.[19] Further, *in vitro*-digested proteins from three different insects have been proposed to exhibit antioxidant and anti-inflammatory effects.[20] Additionally, in a human intervention study, cricket consumption was found to improve gut health and reduce systemic TNF-α levels;[21] while other studies also indicated improvements in microbiota and metabolic benefits in animals after the consumption of mealworms.[22,23] Collectively, this evidence suggests potential health benefits associated with insect consumption, particularly in the context of intestinal tract and inflammatory response.[24]

However, a current concern about insect consumption is the potential allergic effects on sensitized individuals or those allergic to other inhalant or food allergen sources such as house dust mites or crustaceans.[24,25] The yellow mealworm and lesser mealworm belong to the Insecta class, one of the four subphyla of Arthropoda. In relation to arthropods, several allergens have been reported, including tropomyosin,[26] arginine kinase,[27] chitinases[28] and glutathione *S*-transferase,[29] that affect humans through cross-reactivity. However, studies in this context suggest that the allergenicity of edible insects is species-specific and that thermal processing may partially reduce cross-allergenicity.[30] In the context of insect mealworm protein, individuals allergic to shrimps exhibited cross-reactivity due to high protein homology across different arthropod subphyla,[31] while one case report that documented food-induced anaphylaxis to *T. molitor* in a patient allergic to dust mites attributed the allergic response to α-amylase, tubulin, and larval cuticle proteins.[32]

The origins of allergic diseases have traditionally been explained by immunoglobulin E-mediated immune responses to account for asthma, atopic dermatitis, atopic rhinitis, and food allergy. Research insights into disease origins support a broader array of factors that predispose, initiate, or exacerbate altered immunity in allergic diseases, such as (1) inherent epithelial barrier dysfunction; (2) loss of immune tolerance; (3) disturbances in the gut; and (4) organ-specific microbiomes, diet, and age.[33] Here, we focus on the gut barrier function as a key factor in preventing or facilitating food allergy. In fact, Galli *et al.* previously reported that epithelial barrier deficiencies often lead to a state of constant inflammation that makes tissue repair difficult. Deficient barrier integrity facilitates allergen entry and lowers the threshold for sensitization to innocuous substances because of the inflammatory environment, and therefore likely precipitates allergic sensitization at distal organs.[34]

Although the consumption of *Alphitobius diaperinus* and *Tenebrio molitor* has raised concerns about their potential to induce food allergic reactions in sensitized or allergic individuals, research into the specific health impacts of these insects, including both their detrimental and beneficial effects, is still limited and further studies are needed to understand their role in inflammation and intestinal dysfunction.

Thus, this paper analyses how insect flours interacted with the gastrointestinal tract in the human intestine *ex vivo* and how this interaction might influence the response of the whole organism in rats, focussing on the inmunologic response. From a more holistic view of allergy development, we evaluated how the effects of low-dose insect supplementation depend on disease status by comparing the effects on a healthy animal *vs.* a model of LPS-induced inflammation and intestinal barrier dysfunction.

## Experimental methods

### *Ex vivo* study with human colonic explants exposed to *in vitro* digested insect, beef and almond

Buffalo (*A. diaperinus*) powder and insect protein concentrate powder (IPC) from *A. diaperinus* (Protifarm, Belgium), a lean portion of beef (*Bos primogenitors*) from the Central Market in Tarragona, Spain, and almond (*Prunus dulcis*) flour from Borges Agricultural & Industrial Nuts (BAIN) were used for human colon treatments. Samples were stored in the dark at −20 °C until use.

Buffalo, IPC, beef and almond food powders were then *in vitro* digested according to the INFOGEST harmonized protocol.[35] This digestion involved simulating *in vivo* digestion in oral, gastric and intestinal stages *in vitro* using commercial enzymes such as amylase, pancreatin and trypsin and inactivating the enzymes. The detailed procedures and characterization of the digestion products have been described previously.[36]

To check the effectiveness of enzymatic digestion, SDS-PAGE was performed with the samples obtained.[37]

For the explant culture, healthy viable colon tissues were obtained from 10 patients (average age 65 years) who had undergone colon surgery. All donor patients met the study criteria and gave their informed consent. The exclusion criteria were anti-inflammatory drug use, alcohol abuse, and intestinal disease. The study was approved by the Clinical Research Ethics Committee (CEIC) of the Hospital Universitari Joan XXIII in Tarragona (CEIm 101/2017).

Tissues obtained from the proximal and distal colon were transferred to the culture laboratory within 30 min in cold oxygenated Krebs–Ringer buffer (pH = 7.4) with D-mannitol 10 mM.[38]

The excised samples were cut up to generate six explants from each human donor. The 5 mm diameter explants were placed in a 48-well plate pre-filled with 400 µL of KRB buffer with D-mannitol, and divided into five treatment groups: control, beef, almond, insect, and IPC. The control group was treated with the same KRB buffer with glucose. The digested samples were adjusted to a dose of 5 mg protein per mL. The medium was collected after 30 minutes of incubation and stored at −80 C.

The secretion of human inflammatory cytokines and intestinal immunoglobulins TNF-α, IL-10, IL-8, IgE and sIgA was quantified using Enzyme-linked Immunosorbent Assay (ELISA) with colorimetric detection. The overall experimental procedure is shown in Fig. 1. For immunosecretome analysis in human colon explants, intestinal secretion of IL-10, IL-1β, IgE and sIgA was measured with the Elabscience ELISA kit (Texas, United States). The human TNF-α ultrasensitive ELISA kit was obtained from Thermofisher (Invitrogen, Barcelona) (Cat. no: KHC3014). Colon IL-8 secretion was measured with Millipore (Sigma Aldrich, Madrid) (Cat. no: RAB0319). All immunomarkers were measured according to manufacturers' instructions.

### *In vivo* study of the consumption of *A. diaperinus* and *T. molitor* flour by Wistar rats

Buffalo (*A. diaperinus*) flour was obtained from Protifarm NV (Ermelo, Gelderland, The Netherlands) with a caloric content of 6.1 kcal $g^{-1}$ and a macronutrient composition of 56.31% protein, 18.82% fat, 7.44% fibre, and 6.3% carbohydrates (1.30% starch).

*T. molitor* flour from larvae was prepared from insects purchased from a local supplier (Iberinsect, S.L; Reus, Spain). Insect flour was prepared and processed by the FoodIE Research Group and the Mobiofood Research Group at Universitat Rovira i Virgili (URV), Spain. The composition of *T. molitor* flour was 56.10% protein, 26.31% lipids and 7.78% carbohydrates (3.34% starch), as measured by AGROLAB, S.A. The caloric content was determined to be 6.23 kcal $g^{-1}$ by the Institute of Agrifood Research and Technology (IRTA, Catalonia, Spain).

The microbial content of *A. diaperinus* flour was determined at Protifarm (Ermelo, Gelderland, The Netherlands). The microorganism content of *T. molitor* flour was analysed at AGROLAB S.A (Tarragona, Spain). Both microbiological analyses showed levels of bacteria, moulds and yeasts absent or below the toxicity levels for both insects.

Forty, six-week-old, Wistar female rats (Janvier, Castellar del Vallès, Spain) were included in the *in vivo* study. The animals spent an adaptation period of 14 days at the Universitat Rovira Virgili animal facility under standard conditions. They were caged in pairs at 22 °C with a standard 12-hour light–dark cycle, ventilation, *ad libitum* access to tap water, and a standard Teklad diet (Cat no: Teklad 2014, Envigo++, Barcelona, Spain) consisting of 20% protein, 13% fat and 63% carbohydrates. The rats' standard diet was a plant-based protein maintenance diet with 4.073 kcal $g^{-1}$, as measured by IRTA, Catalonia, Spain.

After this adaptation period, the animals were individualized and randomly divided into five experimental groups, with identical initial body weight, each of which was given a different treatment. Lipopolysaccharide (LPS) treatment was given, based on the body weight (BW) of the rats, to induce intestinal immune disruption. LPS was obtained from *E. coli* serotype O111.B5 (Merck Lifesciences, Madrid, Spain; Cat no: 4357765). As detailed in Miguéns-Gómez *et al.*,[39] the treatment lasted 26 days (also shown in Fig. 2) and involved five groups as follows:

Group 1 comprised animals that received a standard (STD) diet *ad libitum* (C group);

Group 2 comprised animals that received an STD diet plus five intraperitoneal (i.p.) doses of LPS at 0.5 mg $kg^{-1}$ of BW on the last five days (C + LPS group);

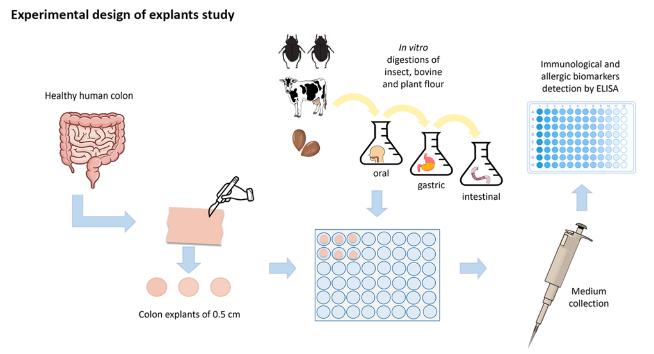


**Fig. 1** Graphical representation of the experimental design of the explant's study.

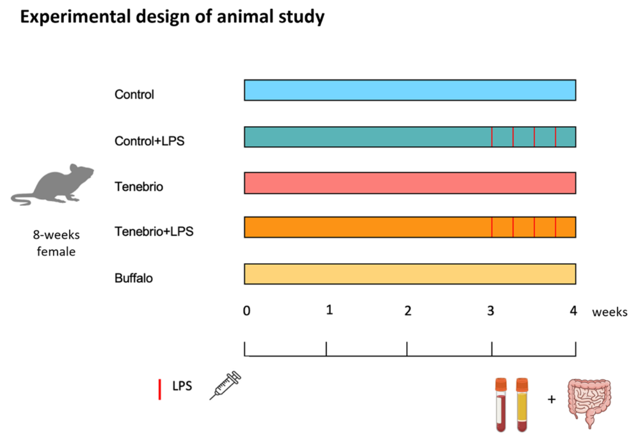


**Fig. 2** Experimental design of Wistar rat study.

Group 3 comprised animals that received an STD diet plus a daily oral dose of *T. molitor* flour (300 mg of protein per kg BW per day) (T group);

Group 4 comprised animals that received STD diet plus a daily oral dose of *T. molitor* flour (300 mg of protein per kg BW per day) and an i.p. dose of LPS 0.5 mg per kg of BW on the last five days (T + LPS group); and

Group 5 comprised animals that received STD diet plus a daily oral dose with *A. diaperinus* flour (300 mg of protein per kg BW per day) (Buffalo group).

The insect flours were administered by controlled voluntary oral intake with a syringe, at a dose of 300 mg of protein per kilogram of body weight dissolved in water, at 6 pm. The treatment thus consisted only of raw insect meal mixed with tap water, while the control groups received the equivalent volume of tap water.

During the experiment, the health status of the animals was regularly monitored for cleanliness, general physical appearance, faecal consistency, and stress symptoms (hair loss, lack of appetite, *etc*.).

After 21 days, the animals were euthanized *via* exsanguination under anaesthesia, administered at a dose of 100 mg per kg BW of pentobarbital. Some of the rats' main organs (the duodenum, jejunum and ileum from the small intestine; the distal and proximal large intestine; and the thymus, spleen, kidney and liver) were excised, weighed and frozen immediately in liquid nitrogen for future analysis. White abdominal adipose tissue (WAT) from mesenteric, retroperitoneal and epididymal locations was excised and weighed to calculate the percentage of adiposity. Blood was collected with ethylenediaminetetraacetic acid (Deltalab, Barcelona, Spain) as anticoagulant. Plasma was obtained by centrifugation at 1500*g* for 15 min at 4 °C and frozen immediately at −80 °C for future parameter quantification. All procedures were approved by the GENCAT Animal Experimentation Committee (number 11701).

### Biochemical analysis

Colorimetric kits from QCA, (Tarragona, Spain), Materlab (Madrid, Spain) and Wako (Kyoto, Japan) were used to determine the following plasma biochemical parameters: cholesterol (QCA, Ref. 995282), glucose (QCA, Ref. 998282), triacylglycerols (QCA, Ref. 992330), urea (QCA, Ref. 993648), creatinine (QCA, Ref. 990310) and ketone bodies (β-hydroxybutyrate), (Materlab, Ref. HB8855).

### Intestinal barrier, immunological and allergenic analyses

Two days before euthanasia, the ovalbumin (OVA) test was run to determine intestinal permeability.[40] These analyses were carried out using Ovalbumin ELISA kits (Cat. no. MBS2000240) from MyBioSource (San Diego, CA, USA).

Plasma levels of total IgE and histamine were measured as biomarkers of allergy at the end of the experimental period. IgE (Cat. no. CN: E-EL-R0517) and histamine (Cat. no. CN: E-EL-0032) kits were purchased from Elabscience (Texas, United States) and performed according to the manufacturer's instructions.

Plasma inflammatory cytokine markers (IL-1β, IL-10, and TNF-α) were determined by ELISA. The ELISA kit for IL-10 (Cat. no. CN: 88-50629) was purchased from Thermofisher (Invitrogen) (Life Technologies, Madrid, Spain). ELISA for TNF-α (Cat. no. E-EL-R2856) and IL-1β (Cat. no. E-ELL-R0012) were purchased from Elabscience.

After the intestine was removed and before the intestinal parts were excised, the intestinal lumen contents were subjected to two lavages with 4 mL of PBS. The intestinal lumen contents of the small and large intestine were obtained separately and frozen immediately at −80 °C for future analysis. Secretory IgA (sIgA) levels were measured in small and large intestinal lavage fluids using the ELISA kit from MyBioSource (Cat. no. MBS9711882).

Total RNA and cDNA were obtained as previously reported.[41] Quantitative PCR amplification was performed using specific TaqMan® probes for the sIgA inducing protein (Rn01406210_s1) and IL-1β (Rn00580432_m1) genes, and PPIA (cyclophilin) (Rn00690933_m1) as reference gene. The relative expression of each gene was compared with the control group using the $2^{-\Delta\Delta Ct}$ method and with the cyclophilin gene as reference.

### Histological analysis of the intestinal sections

One centimetre of each part of the intestine was fixed for 24 h in 4% formaldehyde solution and transferred to 70% ethanol solution for preservation until it was embedded in paraffin blocks. The samples analysed (at the Laboratory of Biology and Pathology of the Endocrine Pancreas of the Unité de Biologie Fonctionnelle et Adaptative, CNRS at Université Paris Cité, F-75013 Paris, France) were the duodenum, jejunum, ileum and ascending colon from five animals from each experimental group.

Samples were cut at 5 mm and placed on glass slides. Haematoxylin and eosin (H&E) staining was then performed following standard procedures.

Using an OLYMPUS BX60 microscope equipped with Histolab 10.5.1 (Microvision Instruments, Evry, France) software for histology and morphometrics counting, the intestinal epithelium was analysed by measuring the villus height, villus width, crypt depth, epithelium height and villus-to-crypt ratio, as described in our previous study.[42] The percentage of goblet cells was also analysed by counting the number of goblet cells and the number of epithelial cells.

### Statistical analysis

Results are presented as means ± SEM. Data analysis was conducted with the XLSTAT 2023 statistical software (Addinsoft, USA). In the *ex vivo* human experiment, group differences were assessed through one-way ANOVA, followed by Tukey HSD multiple comparisons test.

For the rat experiment, statistical differences were analysed using Student's *t* test, comparing each experimental group with the corresponding control group. Specifically, Tenebrio, Buffalo and Control + LPS were compared with Control, while Tenebrio + LPS was compared to Control + LPS.

Statistical significance was considered for mean differences if $p < 0.05$.

## Results

### Insect-derived flours produced a healthier inflammatory secretome in human colonic explants

To assess how the human colonic immune system responds to various protein sources, we exposed healthy human colon explants to digested flours for 30 minutes at a concentration protein that stimulated enterohormone secretions in paired colonic samples.[37]

The levels of proinflammatory protein IL-8 and TNF-α were determined in the media of colonic explants after insect, almond and beef exposure (Fig. 3). IL-8 levels from human explants treated with insect and IPC showed lower inflammatory profiles than those treated with beef or almond (Fig. 3A). Beef also had a proinflammatory effect by inducing an increase in TNF-α levels, whereas neither insect, IPC nor almond treatment led to elevated TNF- α levels (Fig. 3B).

Also assessed was the anti-inflammatory cytokine IL-10, which was significantly higher in explants treated with almond flour but remained unchanged after insect, IPC or beef treatment (Fig. 3C).

The levels of sIgA showed the extent of protection from intestinal pathogens in the intestinal lumen. No treatment showed changes in sIgA levels with respect to the control (Fig. 3D).

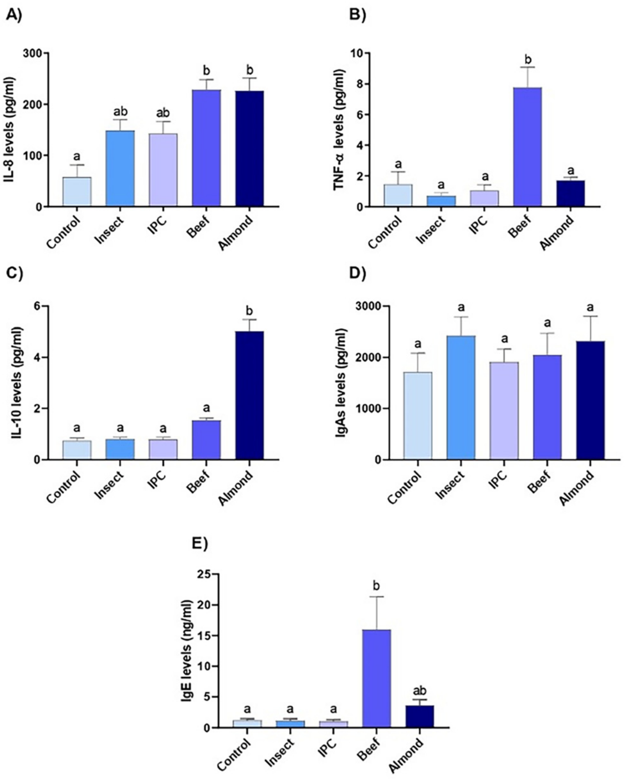


**Fig. 3** Effect of different digested proteins (5 mg protein per mL) on levels of IL-8 (A), TNF-α (B), IL-10 (C), sIgA (D) and IgE (E) in human colon explants. Results are represented as mean ± SEM. *n* = 10 humans per group. We used ANOVA and Tukey multiple comparisons test. *P* values < 0.01. Different letters indicate significant differences.

Also determined was the level of allergy-related IgE immunoglobulin (Fig. 3E). Neither whole insect nor IPC from *A. diaperinus* showed a similar allergenic profile to that of almond extract, whereas beef induced a significant increase in IgE levels.

### Whole-health status of insect-fed rats

To analyse the effects on an *in vivo* system, we worked with two animal models, one of which was a healthy rat and the other was a model of LPS-induced mild inflammation and intestinal dysfunction.[43] This LPS-induced disease model wants to mimic all pathologies that presented an altered physical barrier and a proinflammatory intestinal environment in the small intestine which would be the best candidates to be sensitive to this atypical food component.[44,45] Using these animal models, we tested two species of insects to detect potential species-specific effects.

In the healthy rat model, after 21 days of insect administration no animal showed any physical sign of stress (gastrointestinal, respiratory, or skin alteration) during the nutritional intervention. Treatment with buffalo flour did not change the weight of any organs in comparison with those of the control. Supplementation with *T. molitor* insect flour produced a trend towards an increase in % body weight gain but not in adiposity and did not change the weight of the thymus, liver, stomach, spleen, or kidney. There were no differences in total intestinal length between the groups (Table 1). In healthy rats, the ingestion of insect flour did not modify glucose, triglycerides (TAGs) or total cholesterol (Table 2). The levels of ketone bodies were undetectable and urea and creatinine in plasma were unchanged by insect consumption.

In line with the results in humans, in this healthy model the administration of insect protein (Buffalo or Tenebrio flour) did not alter the levels of the proinflammatory cytokines IL-1β and TNF-α. Similarly, no changes were observed in IL-10.

In the intestinal dysfunction model, the weights of the liver and spleen in the LPS group clearly increased while that of the thymus decreased (Table 1). *Tenebrio molitor* ingestion in LPS-

**Table 1** Organ weights of healthy Wistar female rats and LPS-Wistar inflamed rats after daily ingestion of *T. molitor* or *A. diaperinus* for 21 days

| Organ (g) | Control | Tenebrio | Buffalo | LPS | LPS + Tenebrio |
|---|---|---|---|---|---|
| Liver | 7.46 ± 0.35 | 7.86 ± 0.19 | 8.06 ± 0.25 | 8.91 ± 0.37* | 9.41 ± 0.28 |
| Stomach | 1.38 ± 0.07 | 1.42 ± 0.04 | 1.41 ± 0.03 | 1.33 ± 0.03 | 1.37 ± 0.05 |
| Kidney | 0.34 ± 0.01 | 0.31 ± 0.01 | 0.32 ± 0.01 | 0.34 ± 0.01 | 0.34 ± 0.01 |
| Thymus | 0.49 ± 0.04 | 0.57 ± 0.05 | 0.52 ± 0.10 | 0.33 ± 0.03* | 0.49 ± 0.06$^{\$}$ |
| Small Intestine length (cm) | 97.6 ± 0.57 | 100.1 ± 0.17 | 98.0 ± 0.44 | 96.2 ± 0.68 | 100.6 ± 0.52 |
| Spleen | 0.84 ± 0.05 | 0.84 ± 0.03 | 0.86 ± 0.04 | 1.53 ± 0.10* | 1.49 ± 0.13 |
| % BW gain | 9.42 ± 1.03 | 13.98 ± 2.83$^{\#}$ | 9.80 ± 1.04 | 7.01 ± 1.40 | 6.64 ± 1.26 |
| % Adiposity | 4.38 ± 0.25 | 5.12 ± 0.59 | 4.26 ± 0.26 | 4.12 ± 0.21 | 4.37 ± 0.49 |

*n* = 7–8 animals per group. Data are mean ± SEM. Student's *t* test was performed. * indicates $p < 0.05$ *vs.* control group; $^{\#}$ indicates $p < 0.1$ *vs.* control group; $^{\$}$ $p < 0.05$ *vs.* LPS.

**Table 2** Biochemical markers of metabolic status in healthy and LPS-inflamed female Wistar rats with a daily insect intake of *Tenebrio molitor* or *Alphitobius diaperinus* for 21 days

| Plasma | Control | Tenebrio | Buffalo | LPS | LPS + Tenebrio |
|---|---|---|---|---|---|
| Glucose (mM) | 7.18 ± 0.15 | 7.21 ± 0.38 | 7.35 ± 0.14 | 6.44 ± 0.24* | 7.09 ± 0.16$^{\$}$ |
| TAGs (mM) | 0.18 ± 0.02 | 0.22 ± 0.05 | 0.17 ± 0.02 | 0.18 ± 0.03 | 0.20 ± 0.01 |
| Cholesterol(mM) | 0.45 ± 0.01 | 0.46 ± 0.03 | 0.48 ± 0.03 | 0.35 ± 0.02* | 0.37 ± 0.02 |
| Urea (mM) | 3.20 ± 0.21 | 2.93 ± 0.13 | 3.28 ± 0.13 | 1.70 ± 0.18* | 2.11 ± 0.13$^{\$}$ |
| Creatinine (μM) | 2.68 ± 0.49 | 2.59 ± 0.28 | 3.18 ± 0.24 | 2.64 ± 0.56 | 3.43 ± 0.34 |

Plasma levels of glucose, triglycerides, cholesterol, urea and creatinine at the end of the study. $n = 7–8$ animals per group. Data are mean ± SEM. Student's *t* test was performed. * indicates $p < 0.05$ *vs.* control group; $^{\$}$ $0.05 > p < 0.1$ *vs.* LPS.

treated animals slightly prevented the decrease in thymus weight compared to the LPS group. The disease model presented mild alterations in some biochemical parameters (Table 2). LPS treatment reduced the plasma levels of total cholesterol, glucose and the urea. However, TAGs and creatinine remained unchanged. Interestingly, ingestion of *T. molitor* seemed to prevent the decrease in blood glycemia and uraemia.

### Effects of insect consumption on intestinal permeability, immune barrier and allergenicity

Both LPS-treated groups showed an increase in intestinal permeability compared to controls, though insect supplementation did not exert any significant effect with this time and dose (Fig. 4a). In healthy rats, insect supplementation showed no change in intestinal permeability compared to controls (Fig. 4a).

To evaluate the effect of insect protein consumption in a pro-inflammatory environment, we measured the serum levels of TNF-α, IL-1β and IL-10. Fig. 4c shows that treatment with LPS significantly increased the levels of TNF-α and increased Il-10 and IL-1β almost three-fold (Fig. 4b and d) in comparison with the control group. Treatment with *T. molitor*, on the other hand, did not modify the secretion of these cytokines.

To assess the health and functionality of the mucosal immune system, we quantified the secretion of secretory immunoglobulin A (sIgA) at the intestinal level. In the healthy model, the results showed that more sIgA was secreted in the small intestine than in the colon (Table 3). Ingestion of the Buffalo and Tenebrio flours alone had no effect on sIgA levels in either the small intestine or the colon.

In the disease model, when comparing the LPS group with the Control group, the statistical analysis indicates a tendency to increase. However, animals that received both LPS injection and consumed Tenebrio did not show significant differences or a trend, neither with the Tenebrio group nor the LPS group. Intraperitoneal LPS administration had local immunology effects in the small intestine but not in the colon. We also analyzed relative expression in the ileum of slgA activation protein (Table 3) but observed no significant changes in gene expression in either the healthy or the LPS-induced inflammatory group.

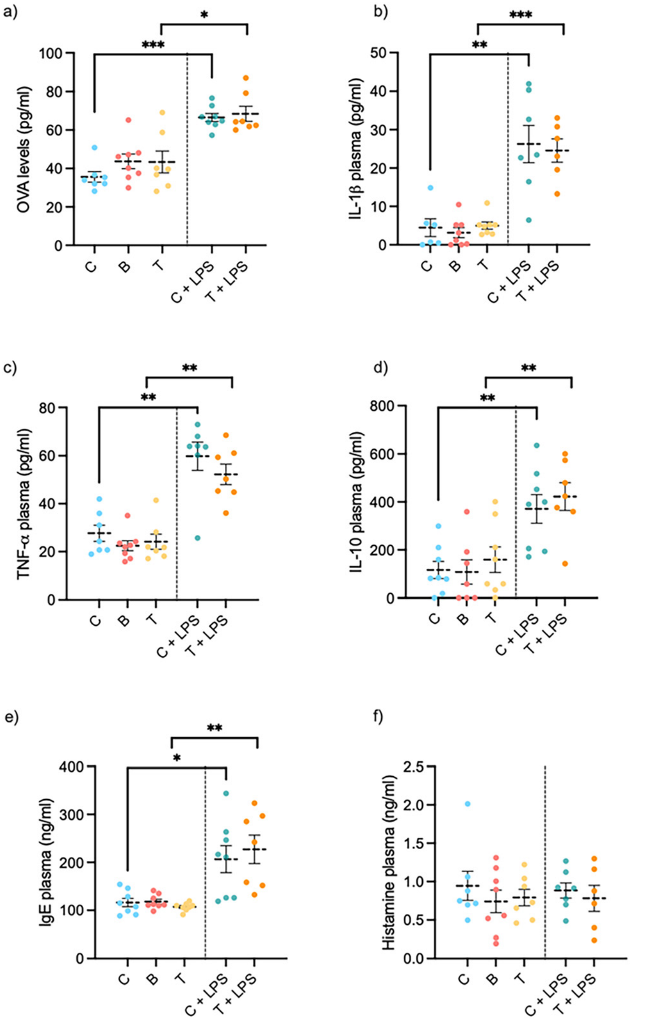


**Fig. 4** Changes in intestinal permeability ((a) OVA test), plasma systemic inflammation ((b) IL-1β, (c)TNF-α and (d) IL-10), allergenic response ((e) IgE and (f) histamine) after chronic doses of insect protein flour in in health and in LPS-induced inflammation female rats. Animals ($n = 7–8$ per treatment) were treated for 21 days. Experimental groups are (C) standard diet; (C + LPS) Control diet plus i.p. LPS (0.5 mg per kg BW) for the last five days; (T) 300 mg protein per kg BW daily supplement of *Tenebrio molitor* flour; (T + LPS); 300 mg protein per kg BW daily supplement of *Tenebrio molitor* flour diet plus i.p. LPS (0.5 mg per kg BW) for the last five days; (B) 300 mg protein per kg BW daily supplement of *Alphitobius diaperinus* flour. *, ** or *** correlation's *p*-value <0.05, 0.01 or 0.001, respectively, denotes statistical significance by Student's *t* test.

**Table 3** Secretory immunoglobulin A (sIgA) at the intestinal level

| | Control | Tenebrio | Buffalo | LPS | LPS + Tenebrio |
|---|---|---|---|---|---|
| Small intestine sIgA (μg ml$^{-1}$) | 9.94 ± 1.25 | 8.00 ± 0.63 | 8.65 ± 1.37 | 17.2 ± 3.49$^{\#}$ | 13.6 ± 1.95 |
| Ileal IgA i.p. gene expression | 0.91 ± 0.14 | 1.19 ± 0.30 | 0.87 ± 0.07 | 1.04 ± 0.19 | 0.76 ± 0.20 |
| Colonic sIgA (μg ml$^{-1}$) | 1.27 ± 0.09 | 1.19 ± 0.08 | 1.17 ± 0.05 | 1.13 ± 0.05 | 1.23 ± 0.08 |

$n = 7–8$ animals per group. Data are mean ± SEM. Student's *t* test was performed. $^{\#}$ indicates $0.05 > p < 0.1$ *vs.* control.

To evaluate potential *in vivo* sensitization to the consumption of *A. diaperinus* or *T. molitor* insect flour for 21 days, IgE and histamine plasma biomarkers were tested. In the healthy

**Table 4** Morphometric intestinal variables affected by insect consumption in healthy and LPS-Wistar rats

| Small intestine | Control | Tenebrio | Buffalo | LPS | LPS + Tenebrio |
|---|---|---|---|---|---|
| **Duodenum (µm)** | | | | | |
| Villus length | 548.13 ± 31.24 | 616.33 ± 29.62 | 597.29 ± 23.19 | 558.27 ± 25.37 | 549.71 ± 34.04 |
| Villus width | 78.48 ± 3.27 | 79.57 ± 2.43 | 76.21 ± 3.03 | 83.32 ± 2.88 | 79.80 ± 2.43 |
| Crypt depth | 168.94 ± 8.63 | 196.92 ± 17.41 | 187.20 ± 10.81 | 191.89 ± 17.18 | 181.79 ± 9.71 |
| Crypt width | 71.25 ± 4.42 | 71.05 ± 2.77 | 74.86 ± 4.77 | 78.86 ± 5.83 | 73.85 ± 2.31 |
| Epithelium w | 777.34 ± 22.76 | 848.24 ± 54.28 | 854.31 ± 48.23 | 803.30 ± 40.45 | 791.76 ± 45.40 |
| Villus/crypt ratio | 3.34 ± 0.45 | 3.50 ± 0.67 | 3.20 ± 0.08 | 3.01 ± 0.32 | 3.06 ± 0.24 |
| M (surface ratio) | 8.4 ± 0.12 | 9.4 ± 0.52 | 8.79 ± 0.46 | 7.93 ± 0.60 | 8.17 ± 0.52 |
| **Jejunum (µm)** | | | | | |
| Villus length | 404.72 ± 14.96 | 407.33 ± 14.02 | 414.78 ± 12.81 | 393.50 ± 26.82 | 366.32 ± 17.30 |
| Villus width | 55.28 ± 2.74 | 55.86 ± 5.69 | 53.77 ± 5.20 | 58.14 ± 2.68 | 52.73 ± 5.39 |
| Crypt depth | 168.94 ± 8.63 | 196.92 ± 17.41 | 187.20 ± 10.81 | 191.89 ± 17.18 | 181.79 ± 9.71 |
| Crypt width | 58.34 ± 4.42 | 58.44 ± 5.54 | 51.92 ± 3.79 | 56.76 ± 3.00 | 54.76 ± 2.49 |
| Epithelium w. | 586.74 ± 24.38 | 576.25 ± 20.85 | 588.07 ± 18.35 | 576.25 ± 20.85 | 533.05 ± 22.47 |
| Villus/crypt ratio | 2.90 ± 0.03 | 3.37 ± 0.12* | 3.22 ± 0.15& | 3.28 ± 0.33 | 3.00 ± 0.05 |
| M (surface ratio) | 7.8 ± 0.43 | 7.96 ± 0.67 | 8.8 ± 0.51 | 7.7 ± 0.57 | 7.45 ± 0.39 |
| **Ileum (µm)** | | | | | |
| Villus length | 269.50 ± 21.89 | 282.90 ± 5.14 | 248.96 ± 16.71 | 244.04 ± 10.28 | 261.89 ± 11.79 |
| Villus width | 55.92 ± 6.38 | 46.60 ± 2.03 | 47.93 ± 7.06 | 53.98 ± 5.08 | 47.16 ± 1.33 |
| Crypt depth | 102.55 ± 7.06 | 100.05 ± 5.51 | 103.73 ± 3.39 | 105.62 ± 3.13 | 104.95 ± 3.64 |
| Crypt width | 58.14 ± 1.98 | 54.08 ± 2.60 | 55.74 ± 1.43 | 53.10 ± 2.79 | 51.49 ± 1.67 |
| Epithelium w | 408.46 ± 24.50 | 429.35 ± 12.06 | 381.82 ± 20.50 | 374.73 ± 16.02 | 397.30 ± 15.42 |
| Villus/crypt ratio | 2.61 ± 0.15 | 2.85 ± 0.13 | 2.41 ± 0.20 | 2.21 ± 0.01* | 2.49 ± 0.05$^{\$}$ |
| M (surface ratio) | 5.41 ± 0.52 | 6.02 ± 0.29 | 5.20 ± 0.20 | 5.28 ± 0.05 | 5.87 ± 1.21 |
| **Large intestine** | **Control** | **Tenebrio** | **Buffalo** | **LPS** | **LPS + Tenebrio** |
| **Colon (µm)** | | | | | |
| Crypt depth | 113.76 ± 15.40 | 103.56 ± 8.61 | 106.84 ± 7.06 | 106.13 ± 6.58 | 95.22 ± 6.27 |
| Crypt width | 40.95 ± 1.35 | 40.75 ± 1.50 | 38.55 ± 2.27 | 39.46 ± 1.53 | 39.47 ± 1.69 |

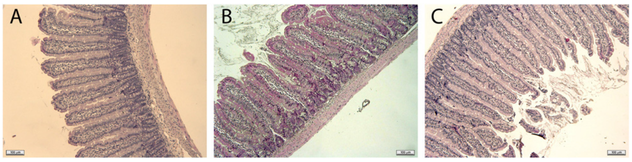
$n$ = 7–8 animals per group. Data are mean ± SEM. Student's $t$ test was performed. * indicates $p < 0.05$ *vs.* control group; $^{\$}$ $p < 0.05$ *vs.* LPS; & $0.05 > p < 0.1$ *vs.* control.

model, neither the histamine nor the IgE levels showed any significant change between groups when the animals received insect flour (see Fig. 4f). In the disease model, IgE plasmatic levels increased significantly compared to controls, while histamine levels remained unchanged after insect consumption.

### Intestinal morphometry in healthy and LPS-Wistar rat models under insect supplementation

To evaluate any putative micro-gut morphological alterations caused by the consumption of insect flour from mealworm and buffalo, we histochemically analysed the morphometric size of the villus and crypt and determined the total epithelium and surface amplification ratio of all intestinal sections (duodenum, jejunum, ileum and proximal colon) (see Table 4) as well as their Goblet cell percentage. Scheme 1 shows examples of the images used for the analysis.

In healthy animals, our results showed that insect flour consumption did not modify villus width, villus height, crypt depth, villus-to-crypt ratio, epithelium width or the M-surface amplification ratio from any intestinal location. This is an indicator of the absence of a deleterious effect. Only Tenebrio increased the villus-to-crypt ration in the jejunum. No changes in Goblet cell number were observed in any intestinal section .



**Scheme 1** Examples of images obtained of jejunal sections of (A) Control group, (B) Tenebrio group and (C) LPS group. Magnification 10×.

The same parameters were also measured in the inflammatory rat model with and without insect flour supplementation (Table 4). In the inflammatory model, a slight proinflammatory state influenced some parameters, including the ileum villus-to-crypt ratio, which decreased, thus indicating a morphological deleterious effect. Interestingly, *T. molitor* supplementation prevented this effect. No changes in Goblet cell number were observed.

## Discussion

We have investigated the effect of a sustainable source of protein on human explants and Wistar rats, focusing on intestinal immunological effects. In a previous complementary work[39] we analysed their effects in food intake and enterohomone modulation. Specifically, two edible insect species approved for human consumption by EFSA were analysed: *Alphitobius diaperinus* and *Tenebrio molitor.*[47] Here our results showed healthy responses with regard to systemic and intestinal inflammation, allergenic response, and intestinal morphology in rats after chronic insect supplementation in both healthy and intestinal disfunction models. This is the first description of the non-allergenic effect of insect ingestion in a disrupted permeability and intestinal inflammation animal model.

The gastrointestinal tract – the primary barrier against food components – plays a crucial role in host defence and immune response.[46] Some studies have reported that increased permeability of the intestinal epithelium can facilitate the entry of potential food allergens, thus heightening sensitization and allergy risks.[33,47] Notably, the intraperitoneal injection of lipopolysaccharides (LPS) leads to systematic and local alterations, including increased intestinal permeability and exacerbated inflammation.[43,48]

In the context of insect consumption, the potential allergenic response is a significant concern. While most existing research focuses on pre-sensitized individuals or those with allergies to crustaceans and inhalants,[49] our study aimed to advance this field by assessing allergenic responses in the context of disrupted intestinal permeability and inflammation. Our results from the LPS model rats revealed an impaired inflammatory and allergic response, as has previously been described.[43] However, in the group that received insect supplementation, no aggravation of this situation was observed, which suggests that the insect did not exert any allergenic response in this rat model of intestinal dysfunction.

Moreover, by evaluating the response to insect consumption in both healthy and non-sensitized human and rat models, our study offers a comprehensive overview of the allergenic potential of low-dose insect consumption. In this sense, the healthy rats that were chronically supplemented by yellow mealworm or lesser mealworm had a normal profile of secreted inflammatory and anti-inflammatory cytokines, while histamine and total IgE were not altered. Previous studies conducted with Sprague-Dawley rats, with longer oral exposures to insects and maximum doses (300–3000 mg $kg^{-1}$ $day^{-1}$) of mealworm, also found no statistically significant increases in serum histamine or IgE concentrations.[50] Our data with doses of 300 mg $kg^{-1}$ $day^{-1}$ for 21 days in Wistar females corroborate those results.

We also directly compared the human colonic immune response to insect extracts to the response to almond and beef protein sources. Our results indicated that exposure to insect extracts led to a lower secretion of inflammatory cytokines and allergenic immunoglobulin compared to almond or beef extracts. Beef especially appeared to be more proinflammatory, as was evidenced by higher cytokines and IgE levels. On the other hand, the response to almond appeared to be counterbalanced by an anti-inflammatory effect. These findings align with previous *in vivo* studies that suggest that beef consumption can alter gut microbiota and activate inflammatory pathways,[51,52] while almond consumption is known to exert a protective effect against the development of gastrointestinal inflammation.[53] Moreover, while explants treated with beef exhibited higher IgE secretion, these levels are still within the low and normal range for intestinal tissue,[54] which suggests that this is not indicative of an allergic response. All these results support the notion of a healthier inflammatory secretome for insect-derived flours compared to those from almond and beef.

An important effector function of gut-associated lymphoid tissue involves the production and secretion of immunoglobulin A (IgA). This immunoglobulin plays a crucial role in protecting the intestine from pathogens, mainly by limiting their interactions with the epithelial cell monolayer.[55] The transcytosis of dimeric IgA antibodies through epithelial cells is mediated by the polymeric Ig receptor, which results in the release of secretory IgA (sIgA).[56] In this context, local antigen exposure could lead to increased sIgA levels in both the exposed area and the enteric mucosa. Measuring IgA in intestinal lavage fluids is therefore a common method for assessing mucosal immune responses.[55] Our animal study showed a tendency only for increased sIgA secretion in rats administered with LPS. In contrast, neither human explants nor rats treated solely with protein sources showed significant alterations in this intestinal immune marker. This suggests that the consumption of these protein sources, including insects, does not cause major immune disturbances, thereby aligning with findings reported by Stull *et al.*[21]

To continue with the intestinal analysis, our findings also showed that insect supplementation did not affect normal intestinal morphology. The typical gradient of morphometric indices from the duodenum to the ileum was maintained, which corroborates the preservation of physiological gut development and absorption processes.[57] Similar to these results obtained by Biasato *et al.* in chickens, chronic insect consumption did not alter the intestinal morphology. Main modification was in the jejunum, where animals supplemented with insects exhibited a higher villus-height to crypt-depth ratio. This effect has been suggested as adaptative response to expand the surface area for nutrient absorption.[58] Our study does not allow support this hypothesis, because there was not a clear effect on food intake, we saw an increased food intake at the first week, but it was lost afterwards.[39] And the other challenging aspect regarding insects-derived products is their digestibility. In this respect, we have previously shown a similar digestibility of *A. diaperinus* to beef samples.[59] Moreover, a lower ileal villus-to-crypt ratio was observed in rats administered LPS, which confirms the finding from studies on mice that the intraperitoneal injection of LPS affects the integrity of the small intestine.[60] Interestingly, the consumption of *T. molitor* appeared to counteract this reduction in the ileal ratio.

Moreover, our findings reveal a reduction in thymus weight following LPS injections, consistent with other studies that have previously reported the potential for LPS-induced thymic atrophy.[61,62] The thymus is a vital organ of the immune system, and its atrophy could compromise its ability to effectively respond to other infections.[63] Interestingly, our observations suggest that the consumption of *T. molitor* may confer protection to the thymus against changes induced by LPS. Despite not observing other improvements in terms of inflammatory status, this result aligns with the potential anti-inflammatory effects associated with insect consumption, as indicated by existing literature.[24] Furthermore, some authors have proposed that thymus gland dysfunction can be ameliorated through nutritional intervention, involving a balance of macronutrients, micronutrients, and the incorporation of probiotics, indicating thymus-gut connections.[64,65] Thus, the potential of insect supplementation to modulate gut microbiota with prebiotic effects,[66] may be linked to thymus healthy. Further research exploring both the inflammatory protection and the prebiotic effects of insect consumption could contribute to a comprehensive understanding of its impact on inflammation.

Although this study primarily focused on the immune system's response, we also considered whole-health parameters. With regard to the plasma parameters, our study indicates good metabolic tolerance to insect administration, with *T. molitor* intake notably ameliorating LPS-altered glucose and urea levels. Our study does not allow to explain these effects, but we can clearly state that they cannot relate it with improvement of TNF-α or IL-1β, which have been demonstrated to induce hypoglycaemia.[45] We cannot discard results from other studies that have reported improved circulating triglycerides, glucose and cholesterol levels in humans and animals following insect consumption.[1] Our initial screening of plasmatic cytokine levels, known to participate in systemic inflammation and exhibiting increased levels in the LPS situation,[67] revealed that LPS administration alone increased the immunological parameters, but the ingestion of insect flour did not exacerbate this effect. Notably, the LPS-induced increase in IL-10 plasma levels is a typical counter-regulatory response to intraperitoneal insult mediated by T-regulatory cells to resolve inflammation.[68]

Note that the EFSA recently approved the insects used in this study as novel food for human consumption and encouraged further research in this area.[16,17] There is growing consensus on the need for further studies to demonstrate the health benefits of insect consumption and enhance its social acceptance, especially in Western diets, which urgently need to change from a conventional to a more sustainable source of protein.[69] The present study describes, for the first time, the chronic effects of consuming two species of insects on both general and intestinal health in rat and human samples and provides novel insights into the immune response in a healthy and a disrupted intestinal model.

In view of all the evidence in support of adding insects to diets and the encouraging results of this study, to validate these findings further research should involve human participants without known allergies. Long-term studies are needed to fully understand the impact of edible insects on human health. As the food industry explores alternative sources of protein, edible insects present a unique opportunity to improve environmental sustainability while also providing substantial nutritional advantages.

## Conclusions

The human *ex vivo* studies have shown that *A. diaperinus* digested *in vitro* induced a less inflammatory profile than either almond or beef extract in colon samples. Moreover, neither *A. diaperinus* nor *T. molitor* administered chronically in moderate doses to healthy Wistar rats compromised gut morphology, plasma biochemistry profile or immune response. In the LPS-induced intestinal dysfunction model, insect consumption ameliorated glycaemia, uraemia and gut villus-to-crypt ratio. These data reinforce the healthy immunological profile of both species of insect assayed. More insights are needed on the bioactive effects of insect flour, especially with regard to the less studied *Alphitobius diaperinus*.

## Author contributions

HS, FJ and MSC: data curation, formal analysis, investigation; FF: investigation; ERG, JM and XT: methodology; AA, MTB, XT and MP: conceptualization, supervision. AA, MTB, HS: project administration, original draft; MP, XT, JM and AA: resources. All the authors participated in funding acquisition and revised and edited the final document before submission.

## Conflicts of interest

There are no conflicts of interest to declare.

## Acknowledgements

We would like to thank Niurka Llopiz and Rosa M. Pastor for respective technical support.

This research was funded by Diputació de Tarragona and Universitat Rovira i Virgili (URV) grant number DIPTA-007 2020/26 (ESSARPI), MCIN/AEI PID2021-122636OB-I00 by ERDF "A way of making Europe", TED2021-131783B-100 funded by MCIN/AEI/10.13039/501100011033 and by the "European Union NextGenerationEU/PRTR". M. Sierra and H. Segú were funded by the M. Franquès programme of the URV. F. Jalševac was funded by Marie Skłodowska Curie grant agreement no. 945413 and Universitat Rovira i Virgili (URV). Montserrat Pinent and Ximena Terra are Serra Húnter fellows.

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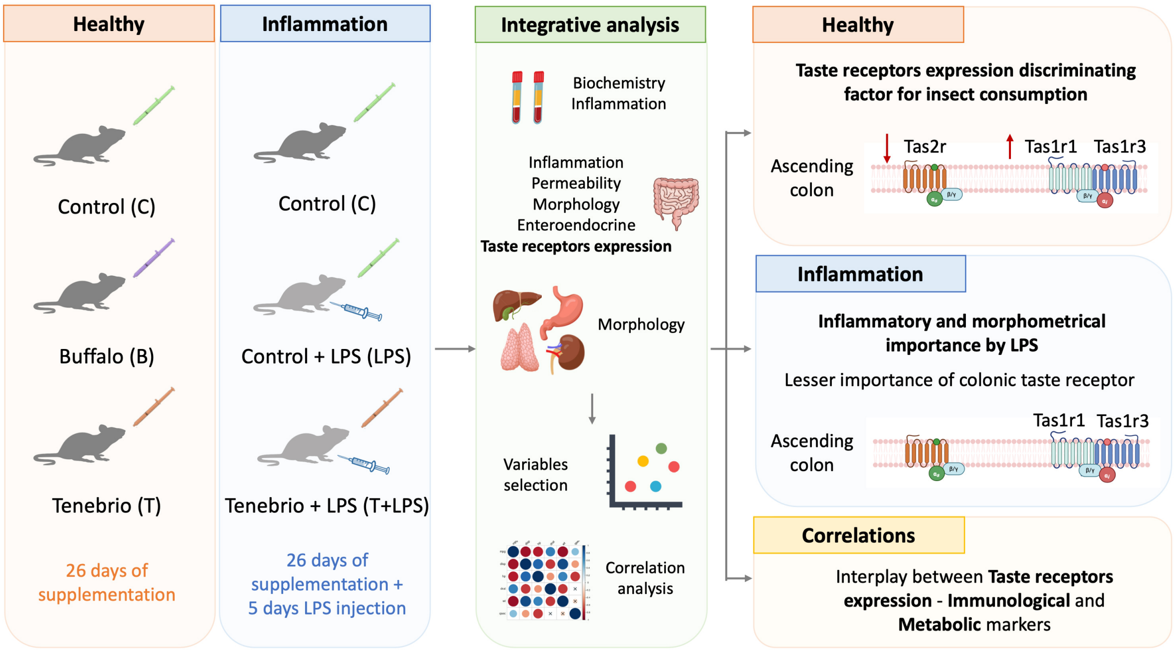
# PART 2

Determination of the impact of chronic low-dose insect protein supplementation on intestinal taste receptor expression and its health implications at both the intestinal and systemic levels, in healthy and intestinal dysfunction models

## Manuscript 2

Intestinal Taste Receptor Expression and Its Implications for Health: An Integrative Analysis in Female Rats after Chronic Insect Supplementation

*Accepted to be published in Journal of Agricultural and Food Chemistry, 20 January 2024*



# Intestinal Taste Receptor Expression and Its Implications for Health: An Integrative Analysis in Female Rats after Chronic Insect Supplementation

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## Abstract

Taste receptors are found in the gastrointestinal tract, where they are susceptible to dietary modulation, a key point crucial for diet-related responses. Insects are a sustainable and a good quality protein source. This study analysed the impact of insect consumption on modulation of taste receptor expression across various segments of the rat intestine under healthy or inflammatory conditions. Female Wistar rats were supplemented with *Tenebrio molitor* (T) or *Alphitobius diaperinus* (B), alongside a control group (C), over 21 days under healthy or LPS-induced inflammation. The present study reveals, for the first time, that insect consumption modulates taste receptor gene expression, mainly in the ascending colon. This modulation was not found under inflammation. Integrative analysis revealed colonic Tas1r1 as a key discriminator for insect consumption (C= 1.04±0.32, T= 1.78±0.72, B= 1.99±0.82, p-value< 0.05 and 0.01, respectively). Additionally, correlation analysis showed the interplay between intestinal taste receptors and metabolic and inflammatory responses. These findings underscore how insect consumption modulates taste receptors, influencing intestinal function and broader physiological mechanisms.

Keywords: taste receptors, insect, umami, bitter, intestinal function, integrative analysis, inflammation.

## Introduction

Taste receptors are predominantly located in taste buds in various places throughout the oral cavity, where they play a crucial role in recognizing exogenous compounds in food and beverages, and other ingested substances. The signals from these receptors are transmitted via afferent gustatory nerves to the brain structures involved in central taste processing [1]. However, these receptors are not limited to the oral cavity as they can also be found in extra-oral tissues and organs throughout the body, from the brain and skin to the reproductive system and gastrointestinal tract [2].

Taste receptors are responsible for identifying diverse tastes, including sweet, bitter, salty, sour and umami. While salty and sour tastes are recognized through ion channels [3], umami, sweet and bitter receptors belong to the G-protein-coupled receptor (GPCR) superfamily and are divided into two types: TAS1R, which are responsible for sweet and umami tastes, and TAS2R, which identify bitter taste. Among type-1 receptors, Tas1r1, Tas1r2 and Tas1r3 operate as heterodimeric duets. Specifically, the Tas1r2-Tas1r3 heterodimer is responsible for recognizing sweetness while L-amino acids and ribonucleotides interact with Tas1r1-Tas1r3, which constitute the taste sensation known as umami [4,5]. With regard to bitter taste receptors, 26 and 37 distinct subtypes have been reported in humans and rats, respectively. However, data regarding the presence of these receptors along the intestine in animal models are still limited. Notably, Tas2r108, Tas2r119, Tas2r138 and Tas2r143 are among the subtypes that exhibit the highest expression levels of all bitter receptors in rats [6]. Moreover, each subtype is susceptible to activation by specific bitter compounds or a broad spectrum of them [7]. However, some of these bitter receptors, including Tas2r108, Tas2r119, Tas2r138, Tas2r139, and Tas2r143, have also been shown to be sensitive to certain peptides and amino acids [8–12]. This study focuses on the modulation of TAS1R and the above mentioned TAS2R expression along the intestine.

The abundance and tissue distribution of taste receptors, especially in the gastrointestinal tract, raises the prospect of their involvement in physiological functions [13]. Since the intestine serves as the primary organ for food digestion, a process that occurs over a relatively extended period, chemosensory receptors in the gastrointestinal wall are subjected to prolonged exposure to agonists and antagonists present in ingested food [14–17]. These components can interact with various receptors, binding and activating them, sending to diverse parts of the organism this information through different kind of signalling molecules. The localization of taste receptors in the enteroendocrine cells plays a role in regulating the secretion of enteroendocrine peptides, including ghrelin, GLP-1, PYY and CCK [4,18–20]. Additionally, several studies have proposed a potential role for taste receptors in the immune function in view of their placement on Goblet and Paneth cells [18,21]. Some researchers have also demonstrated the ability of dietary components to exert a profound effect on the genetic expression of these receptors in the intestine [2,4,16,22]. In this context, exploring how a diet or dietary compound interacts with taste receptors becomes crucial for discerning any potential beneficial or detrimental effects.

Protein is essential for human nutrition as it plays an important role in the formation and repair of tissues and the proper functioning of enzymes and hormones [23,24]. Ensuring sufficient protein intake is crucial for maintaining robust immune function, supporting optimal muscle development, and protecting overall health [25]. Abundant evidence strongly supports the postulation that insects are a source of high-quality protein [26,27], and are considered sustainable sources of protein that require fewer land and water resources than conventional livestock production [27,28]. Based on evaluated studies on the microbiological risk of zoonosis, heavy metal contamination and allergenicity, the European Food Safety Authority (EFSA) has given a favourable opinion on four insects as novel foods, including two mealworms from different beetle species, *Tenebrio molitor* and *Alphitobius diaperinus*, which serve as the focus of study in this article [29–32]. These two insect species, belong to the Tenebrionidae family of the order Coleoptera, highlighting their taxonomic proximity, and the nutritional composition at the larval stage is similar [33]. *T. molitor* has been used across various sectors and in in vivo studies and it is considered one of the most promising insect proteins in the food and feed industries [34]. However, *A. diaperinus* has been less commonly employed, necessitating further studies on their health effects [35]. In this context, some studies have highlighted the bioactive properties of insect peptides, while demonstrating their potential as antihypertensive, anti-inflammatory, antidiabetic, or antioxidant agents [36]. Nevertheless, new studies that provide evidence of the health advantages of insect consumption as well as the precise mechanism of action are still needed. In this sense, prior findings from our research group indicated that chronic low-dose supplementation of *A. diaperinus* in rats decreased local ghrelin levels in the small intestine and increased food intake [33]. Moreover, our research team has conducted several studies to investigate the impact of *A. diaperinus* and *T. molitor* on intestinal immune function and morphology, both in healthy and in inflammatory conditions. Our findings demonstrated healthy responses in terms of systemic and intestinal inflammation, allergenic response and intestinal morphology in rats after chronic insect supplementation in both conditions [37]. However, there is still a lack of evidence for the role of extra-oral taste receptors after insect consumption and its potential implications for intestinal health. Considering the presence of 5-ribonucleotides and several amino acids or peptides specific to insects, these are expected to interact with type I and some type II taste receptors in the intestinal tract, thereby adding a novel dimension to our exploration of the broader impact of insect consumption on intestinal health [38].

Given the evidence supporting potential benefits of insect consumption and the recognized importance of taste receptors in maintaining overall health, our study aimed to explore how dietary components modulate intestinal taste receptors across different health conditions. Specifically, we investigated the broader impact of chronic daily insect supplementation on taste receptor expression in the rat intestine, encompassing both physiological and inflammatory conditions with the main aim of elucidating the complex interplay between dietary interventions and organism responses. Through an integrative analysis approach, we explored the modulation of the expression of intestinal taste receptors by insect protein and their potential role in distinguishing between groups receiving insect supplementation and those that do not. Furthermore, in this study we used an LPS-induced inflammatory model, well-established experimental approach for studying intestinal dysfunction and systemic alterations, including increased intestinal permeability and exacerbation of inflammation [39,40]. Through this model we aimed to gain valuable insights into the mechanisms underlying taste receptor modulation and its relationship to overall health parameters during inflammatory conditions. Additionally, we evaluated the effect of species-specific supplementation, evaluating the most commonly studied mealworm (*T.molitor*) and another less-used larvae (*A. diaperinus*).

## Materials and Methods

### Chemicals

Lipopolysaccharide (LPS) from Escherichia coli O111:B4 (impurities ≤3.00% protein) (Merck Lifesciences, Madrid, Spain; Cat No:4357765). Standard Teklad diet (Envigo++, Barcelona, Spain; Cat No: Teklad 2014). *Tenebrio molitor* flour (Iberinsect, S.L; Reus, Spain), *Alphitobius diaperinus* flour (Protifarm NV, Ermelo, Gelderland, The Netherlands). The nutritional composition of these two insect flours is described in Table 1. TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). Capacity cDNA Reverse Transcription kit (Applied Biosystems, Madrid, Spain), Specific TaqMan® probes (Thermo Fisher Scientific, Madrid, Spain).

Table 1. Nutritional composition of the administered treatments measured on dry matter (values per 100 g insect flour).

| Composition | *A. diaperinus* | *T. molitor* |
|---|---|---|
| Energy (kJ) | 2550 | 2604 |
| Protein (g) | 56.31 | 56.1 |
| Total lipids (g) | 18.82 | 26.31 |
| Starch (g) | 1.30 | 3.34 |
| Fibre (g) | 7.44 | 7.78 |

### Experimental Design

Forty female rats were acclimated for 14 days under standard conditions (22°C with a 12-hour light-dark cycle). During this period, they had ad libitum access to water and were fed a standard Teklad diet (Envigo++, Barcelona, Spain; Cat No: Teklad 2014). After this period of adaptation, the rats were divided into five experimental groups (8 rats per group), categorized into healthy and inflammatory conditions (figure 1). The healthy condition included a control group (Control) fed a standard diet, a group supplemented with *Tenebrio molitor* flour (Tenebrio) (300 mg/kg bw/day), and a group supplemented with *Alphitobius diaperinus* flour (Buffalo) (300 mg/kg bw/day). On the other hand, the inflammatory condition included a control group receiving 5-days intraperitoneal lipopolysaccharide (LPS) injections at 0.5mg/kg of body weight (LPS group), and a group receiving both *Tenebrio molitor* flour and LPS (Tenebrio + LPS group). The intervention lasted for 26 days, and additional details of the experimental design have been described in a previous article [33].

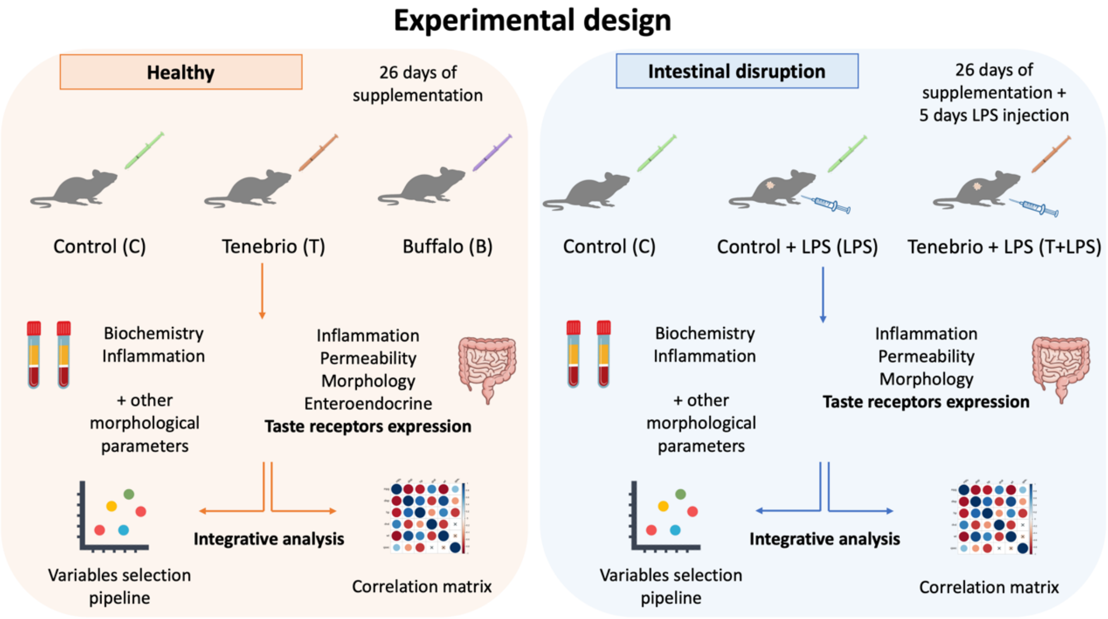

**Figure 1.** Experimental design.

After sacrifice, blood was centrifugated to obtain plasma, while tissue samples, including various intestinal segments (duodenum, jejunum, ileum, ascending colon and descending colon) were rapidly removed, weighed and frozen in liquid nitrogen before storage at -80C until further analysis. After thorough cleaning and removal of fat, samples from the duodenum, jejunum, ileum, ascending colon, and descending colon were demarcated according to the intestinal segment division described by Vdoviaková et al. 2015 [41]. All procedures were approved by the GENCAT Animal Experimentation Committee (number 11701).

## Gene expression of intestinal taste receptors

Total RNA was extracted from the entire tubular tissue from each segment of the intestine using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and following the manufacturer's instructions. Complementary DNA (cDNA) was obtained using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Madrid, Spain), as described in a previous study [42]. Quantitative PCR amplification was performed from a total of 40 ng/μl of cDNA using

TaqMan Universal PCR Master Mix (Applied Biosystems, Madrid, Spain) and specific TaqMan® probes for PPIA (cyclophilin A) (Rn00690933_m1), Tas1r1 (Rn01516038_m1), Tas1r2 (Rn01515494_m1), Tas1r3 (Rn00590759_g1), Tas2r108 (Rn02396427_s1), Tas2r119 (Rn00576950_s1), Tas2r138 (Rn02396417_s1), Tas2r139 (Rn04218919_s1) and Tas2r143 (Rn02585801_s1) genes from Thermo Fisher Scientific. The relative expression of each gene was compared with the control group using the $2^{-\Delta\Delta Ct}$ method [43] and with the cyclophilin gene as the endogenous control gene.

## Statistical analysis

### Univariate analysis

Analyses were performed with XLSTAT 2022 (Addinsoft, USA). A normality test for each group was conducted using the Shapiro-Wilk test. The relative expression of taste receptors is presented in box and whiskers plots, where boxes represent the median and interquartile range and whiskers go down to the smallest value and up to the largest, encompassing the full range of the data. Moreover, the written values are expressed as mean ± standard deviation (SD). Pairwise comparisons for statistical differences were conducted using the Mann-Whitney test. p-values < 0.05 were considered statistically significant. These analyses were conducted in two separated conditions: healthy that included Control, Buffalo and Tenebrio groups; and inflammation condition that included Control, LPS and Tenebrio + LPS. Additionally, the Fold Change of each gene expression was calculated as A/B, with A representing the gene expression mean of the Tenebrio or Buffalo group and B representing the gene expression mean of the control group, when the analysis included the healthy rats. When focused in animal groups under inflammatory situation, the Fold Change was calculated by taking the gene expression mean of the Control or Tenebrio + LPS group as A and gene expression mean of the LPS group as B.

### Integrative Analysis and Variable Selection Pipeline

All data processing, integration, variable selection pipeline and statistical analysis described in this section were performed using RStudio version 2023.03.1 Build 446 (2009-2023 Posit Software, PBC).

Data encompassing morphometric, biochemical, immunological, and intestinal permeability analysis in this study have previously been collected, analysed and used for research purposes [37]. These data include tissue weights, intestinal lengths, and biochemical analyses (including glucose, triglycerides, urea, cholesterol, creatinine and β-hydroxybutyrate). Inflammatory and allergenic parameters in both plasma and intestine (TNF-α, IL-1β, IL-10, secretory IgA, myeloperoxidase (MPO) activity, IgE, histamine, and the relative gene expression of IgA and IL-1β) as well as intestinal permeability. Moreover, results on secretion of the enterohormones (GLP-

1, ghrelin, and insulin), also included in this analysis, have already been published [33]. Additionally, this integral analysis also included the relative gene expression of intestinal taste receptors, which are unique variables to this study and have not been previously used.

All raw data, with medians calculated for missing values and redundant variables removed to reduce data dimensionality and collinearity, were pre-processed. The resulting integrated data consisted of four metabolic variables, six biochemical variables, nine general and twenty-seven intestinal morphometric variables, twelve inflammatory variables and thirty-one TASR gene expressions. The data were centred and scaled using the 'ScaleData' function.

With all the variables, the 'RunPCA' function was employed to conduct Principal Component Analysis (PCA) and determine if samples formed groups or clusters, or if any animal was an outlier that needed exclusion in later steps.

The variables were further analysed in a multivariate approach by our variable selection pipeline, which takes the consensus of three machine learning methods: Elastic Net, Partial Least Squares Discriminant Analysis (PLS-DA), and Random Forest (RF). Elastic Net is useful for dealing with datasets containing a large number of features, some of which are highly correlated. PLS-DA is a versatile statistical method for classifying and discriminating in high-dimensional datasets, which makes it well suited for our objective to distinguish between different experimental groups based on the variables analysed. RF is a powerful, flexible algorithm from the family of tree-based models that can be used for both classification and regression.

Our experimental design encompassed two scenarios: one involving healthy rats and comprising the control, Tenebrio and Buffalo groups, and the other involving rats with induced inflammation and comprising the LPS and Tenebrio + LPS groups. We therefore conducted two separate analyses by applying the algorithms to each scenario and comparing (1) the control group with the Buffalo or Tenebrio group, and (2) the LPS group with the control or Tenebrio + LPS group. With this approach we were able to examine the effects of chronic insect supplementation in both a homeostatic status and an LPS-induced inflammation situation.

Non-zero coefficients from a subset of variables selected from the Elastic Net model, variable importance for projection (VIP) coefficients from the PLS-DA model, and mean decrease Gini values from the RF model were used as measures of variable importance.

Each method generated a set of scores that reflected the importance of variables in relation to the aim of distinguishing between groups. These scores were treated as individual 'scores' for each variable within each method. We then calculated a 'total score' for each variable by summing its 'scores' generated by the three methods. This 'total score' serves as an indicator of the overall importance of the variable within the context of the study. The variable with the highest 'total score' was considered the most important one in consensus. In other words, the variable that was selected by most methods and that achieved the highest score in each was identified as the variable of greatest significance in this study. With these results, an integrative analysis that ranked all variables based on their importance or obtained score was conducted. To refine this ranking and identify the most critical variables for distinguishing between our study groups, we applied the Kneedle algorithm. This algorithm identifies the knee (or elbow) point on the curve formed by the sorted importance scores of the variables while demarcating the most influential variables. This selection method enabled us to focus our subsequent analyses on those variables that displayed the greatest discriminative power between the groups, thus ensuring a more targeted and effective investigation.

A Venn Diagram was created to integrate the two analyses in each case: (1) control versus Buffalo with control versus Tenebrio; and (2) LPS versus control with LPS versus Tenebrio + LPS. This diagram enabled us to determine whether any variable that is crucial in the separation of two groups is also significant in distinguishing one group from a third group. In this context, variables present in the intersection of two ellipses are common to both comparisons. These were then visualized in a Fold Change heatmap that describes how much the variable changes between two conditions as well as the direction of the change.

Finally, a heatmap based on Spearman correlations of taste receptor expression was conducted to explore potential associations between the relative expressions of taste receptors and the other parameters studied in the experiment.

## Results

### Insect consumption primarily modulates taste receptor gene expression in the ascending colon

To address the modulation of the abundances of the main described bitter taste receptors located at the different intestinal locations [6] and umami receptors, we work with the quantification of their mRNA. It allows us to run a quantitative screen of eight of them, as indicative of the potential proteins to be located in the membrane to act as truly receptors for their respective ligands. In the small intestine of healthy female rats, the consumption of both insect species induced no significant changes in the relative expression of the taste receptors assayed when compared with the control group (figure 2 A-C). Taste receptors in the duodenum (figure 2A) were not modified by initially hydrolysed proteins from the stomach. However, the expression of umami taste receptors (Tas1r1 and Tas1r3) in the ascending colon increased when rats were supplemented with either Buffalo or Tenebrio (figure 2D). Note that at this location most protein digestion was completed. A tendency for the Tas1r1 profile to increase was also found in the jejunum when the rats were administered the Buffalo supplement (figure 2B). Finally, with regard to bitter taste receptors, the Buffalo-supplemented group exhibited significantly lower relative expression levels of Tas2r119 and Tas2r138 in the ascending colon than the control group did (figure 2D).

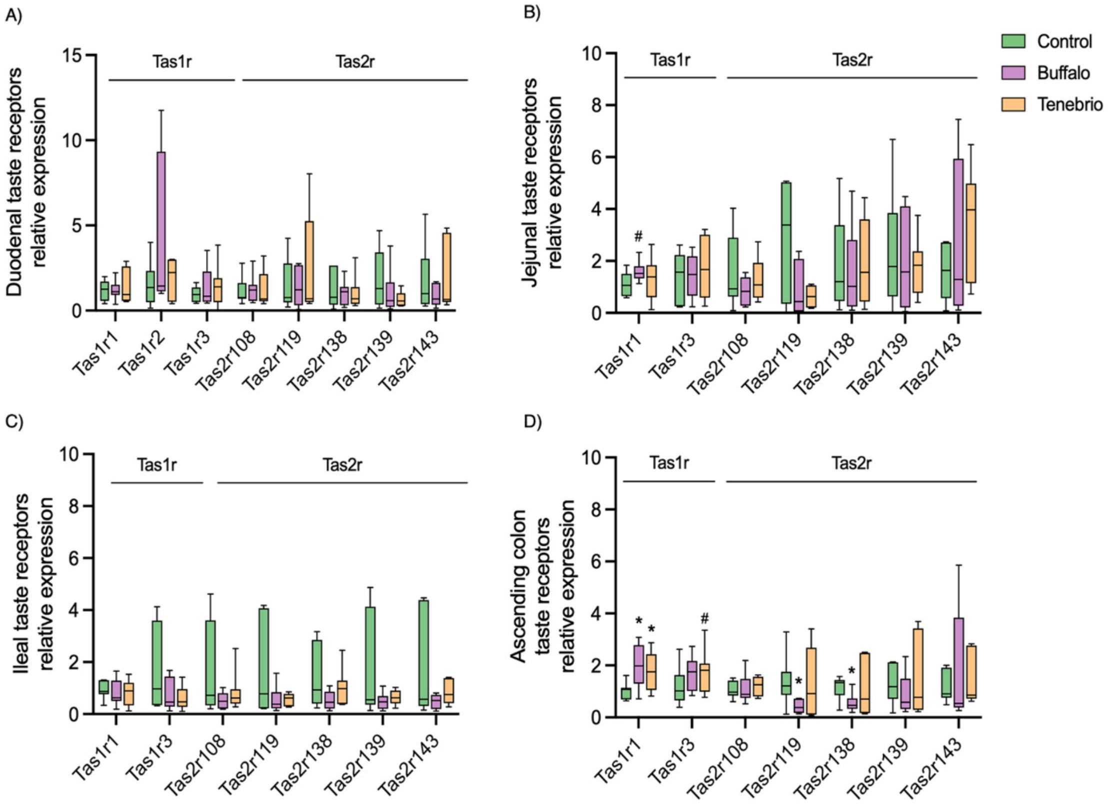

**Figure 2.** Relative expression of intestinal taste recepeptors. Animals supplemented with Buffalo for 21 days are represented in purple, those receiving Tenebrio molitor in orange, and the control group that received water as a vehicle are depicted in green. The data are presented in a box and whiskers plot. Boxes represent the median and interquartile range, while whiskers extend from the smallest to the largest values, encapsulating the full range of the data. Mann-whitney analysis were used to compare each insect-supplemented groups with the control group (n= 7-8/group). * indicates p-value < 0.05 and # indicates 0.05 < p-value < 0.1 compared to control group.

In the descending colon, we measured only Tas1r1 and Tas1r3 receptors. The gene expression of Tas1r1 in the groups under study were similar (control: 1.12 ± 0.44; Buffalo: 1.31 ± 0.73; Tenebrio: 1.36 ± 0.60; p> 0.05). The same pattern was observed with regard to Tas1r3 expression, levels of which between groups were similar (control: 1.06 ± 0.37; Buffalo: 1.31 ± 1.15; Tenebrio: 1.73 ± 0.96; p> 0.05).

## Tas1R1 in the ascending colon is a discriminating factor for insect consumption

To analyse the importance of taste receptors in the intestine for overall intestinal health, we used machine learning algorithms to integrate and identify which variables among the ninety-seven we analysed distinguished between groups most effectively. We conducted this analysis in healthy condition by pairing each insect species with the control group.

Elastic Net, PLS-DA and Random Forest analyses identified twenty-seven key variables that distinguished between the control and Buffalo groups. These variables were then integrated, ranked and visually presented in the Dot Plot shown in Figure 2A. Among these variables a striking representation of taste receptor expression across various segments of the intestine was observed. After applying the Kneedle algorithm to select a subset of the most important of these variables, we selected a total of 11. Among these top variables identified as highly discriminative between groups, the expression levels of certain taste receptors consistently stand out. Specifically, the relative expression levels of Tas1r1 in the ascending colon (Tas1r1 CA) and the jejunum (Tas1r1 J) were consistently identified by all three machine learning algorithms as key variables that effectively distinguish between the control and Buffalo groups (figure 3A). The expression of bitter taste receptor Tas2r119 in both the jejunum and the ascending colon was also identified as a significant variable by two and three methods, respectively.

When data from the control and Tenebrio groups were also subjected to Elastic Net, PLS-DA and Random Forest analyses, nineteen variables were selected (figure 3B). The key discriminative factors again consistently included the relative expressions of taste receptors between these two groups. More specifically, the expression of Tas1r1 in the ascending colon and the expression of Tas2r119 in the jejunum once more emerged as pivotal variables consistently selected by all three methods for differentiation in this comparison, thereby echoing their significance from the previous analysis. To summarize these analyses, the gene expression of Tas1r1 in the ascending colon and that of Tas2r119 in the jejunum indicate that these taste receptors are highly modulated by chronic insect supplementation.

The Venn Diagram (figure 3C) illustrates the number of variables that distinguish the Buffalo or Tenebrio group from the control group and the number of common variables in both insect treatments (represented in the intersection). Of the thirty-five variables that are able to distinguish the insect groups from the control group, eleven were identified as common discriminators. These variables are showcased in the Fold Change Heatmap (figure 3D), which demonstrates the extent of change for each variable comparing Buffalo or Tenebrio supplemented groups and the control group. The colour of each variable indicated that all selected variables exhibit modifications (asterisks indicate those that are statistically significant) in the same direction with insect supplementation, with six of these corresponding to the relative expressions of intestinal taste receptors. Interestingly, general observations across both insect groups were an increase in the expression of colonic umami taste receptors (Tas1r1 and Tas1r3) and a consistent downregulation of some bitter taste receptor expression in the jejunum or ileum. This suggests that insect supplementation could modulate intestinal taste receptors by enhancing colonic umami receptor expression and suppressing some bitter receptor expression. Note also that the expression of Tas1r1 in the ascending colon serves as a pivotal receptor for insect consumption, underscoring its crucial role in discriminating between groups that consume insects and control groups. This significance is evident in both multivariate discriminative analysis and the statistically significant differences observed in univariate analysis between the insect-treated groups and the control group.

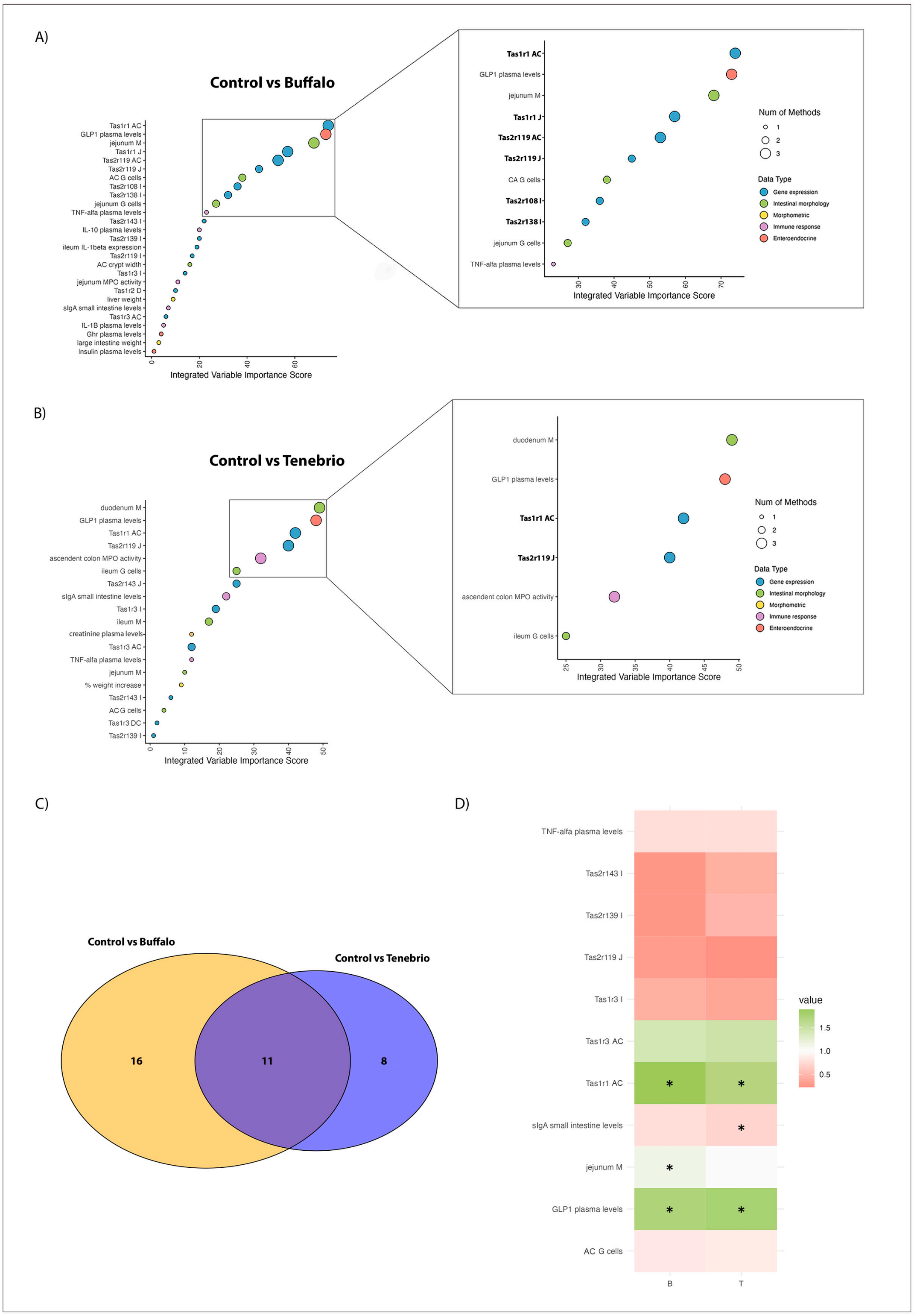

Figure 3. Principal variables distinguishing between the control group and insect-supplemented groups. A) and B) integrate the analysis of selected variables using machine learning algorithms, ranking them to distinguish between Buffalo and Control or Tenebrio and Control groups, respectively. C) Venn Diagram derived from the integrative analysis. D) Fold change Heatmap for the eleven variables that are common in the two comparisons. Green color indicates the gene expression upregulation or higher levels of the variable, while red signifies downregulation or reduced levels, compared to control. * indicates p-value < 0.05. Abbreviations utilized include D (duodenum), J (jejunum), I (ileum), AC (ascending colon), DC (descending colon), G (goblet cells) and M (total absorptive area).

## Modulation of the relative expression of intestinal taste receptors under a proinflammatory stimulus is limited

We have previously shown that LPS-intestinal-induced inflammatory stimulus caused an inflammatory profile at intestinal and peripheral levels that is in some respects ameliorated by insect consumption [37]. In this paper we have investigated the impact of LPS on intestinal taste receptors and explored the effects of insect consumption in this inflammatory scenario. Our results revealed that the injection of LPS had minimal impact on the assessed intestinal taste receptors, with minor significant changes observed. No differences were observed in taste receptor expression in the duodenum (figure 4A). In the jejunum, the relative expression of Tas2r108 was significant lower in the LPS group than in the control group (figure 4B). The expression of taste receptors in the ileum did not change with LPS injection (figure 4C). In the ascending colon, the relative expression of Tas1r1 increased significantly in the LPS group (figure 4D). Similarly, *T. molitor* supplementation in the inflammatory model did not change the expression of taste receptors in any part of the intestine when compared to LPS-treated animals.

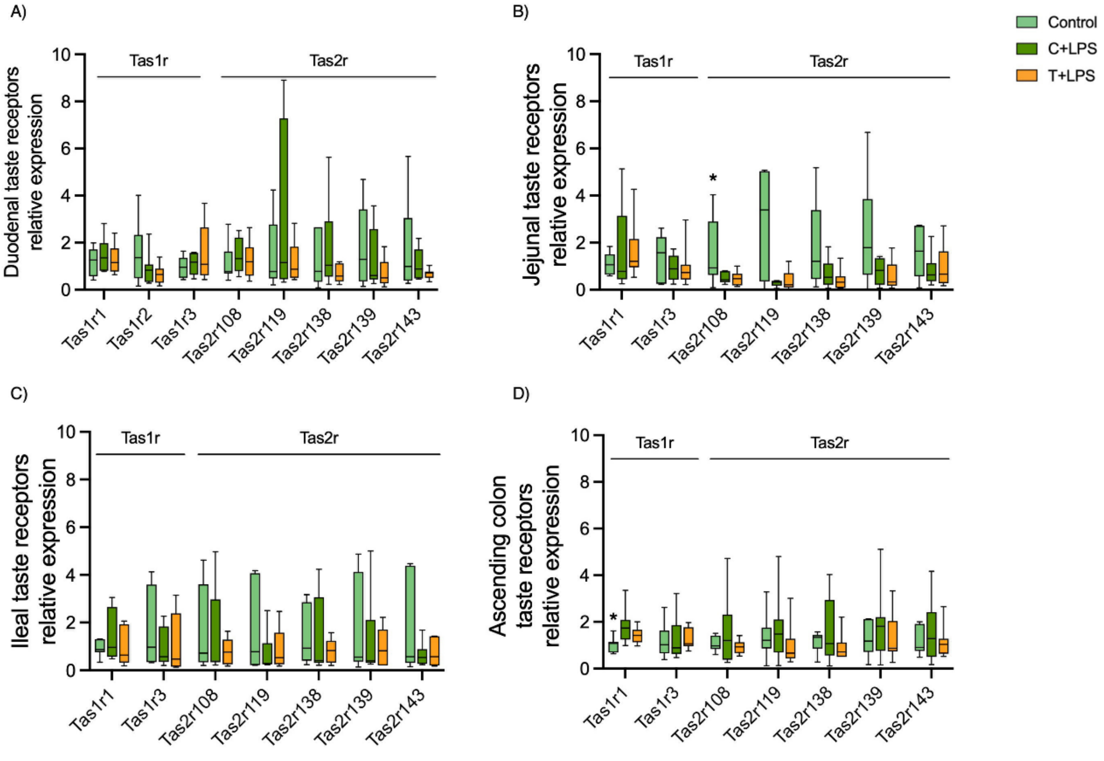

**Figure 4.** Relative expression of intestinal taste recepeptors in LPS groups. Animals were treated by 5-days LPS injection (dark green) or by LPS injection plus Tenebrio molitor (dark orange), or water as the vehicle (green) for 21 days. The data are presented in a box and whiskers plot. Boxes represent the median and interquartile range, while whiskers extend from the smallest to the largest values, encapsulating the full range of the data. Mann-whitney analysis were used to compare Control and T+LPS with LPS group (n= 7-8/group). * indicates $p < 0.05$ compared to LPS group.

The relative expression of Tas1r1 in the descending colon remained unchanged after LPS injection with or without T. molitor administration (control: 1.12 ± 0.44; Tenebrio + LPS: 1.75 ± 0.70; LPS: 1.68 ± 0.61; $p > 0.05$). Tas1r3 expression in the descending colon also showed no significant changes between groups (control: 1.06 ± 0.37; Tenebrio + LPS: 0.97 ± 0.21; LPS: 1.04 ± 0.29; $p > 0.05$).

### The gene expression of colonic taste receptors played a lesser role as key variables for discriminating between rats in an inflammatory state and those in the control group

Integrative analysis revealed that, under proinflammatory stimulus, immunological variables and morphological factors were the main parameters for distinguishing between the control group and the LPS-treated group (figure 5A). However, of the fifty-two selected variables, eighteen taste receptors played a crucial role in this differentiation. Most notably, in the subset of most important variables, the relative expression of taste receptor Tas1r1 in the colon and that of Tas2r119 in the jejunum are also particularly influential in distinguishing between the control group and the LPS-treated group. The lesser importance achieved by taste receptors for discriminating between the LPS-treated group and the control group conditioned the importance of these receptors when the effect on Tenebrio consumption was analysed under this inflammatory status. In the integrative analysis of the relationship between the LPS and the Tenebrio + LPS groups, fewer variables were selected by the machine learning algorithms (figure 5B). However, the relative expressions of colonic umami taste receptors Tas1r1 and Tas1r3, along with the ileal taste receptor Tas2r119, were again selected (but only by one of those algorithms).

When we combined the two integrative analyses in the Venn Diagram, nine of the fifty-two variables that differed between the control group and the LPS groups also differed between the LPS groups (figure 5C). Thus, in this case, the Fold Change heatmap (figure 5D) represents an increase or decrease of a variable with respect to the LPS group. It clearly shows the role of type-I taste receptors in the descending colon for distinguishing between groups. Specifically, Tas1r3 in the ascending colon exhibits a consistent direction in both the control group and the Tenebrio + LPS group, which indicates the potential prevention of LPS-induced alterations. Tas1r1 in the descending colon is also key in separating the LPS group from the control group, where the LPS induced an upregulation of its expression. In this context, insect consumption also increases the relative expression of Tas1r1.

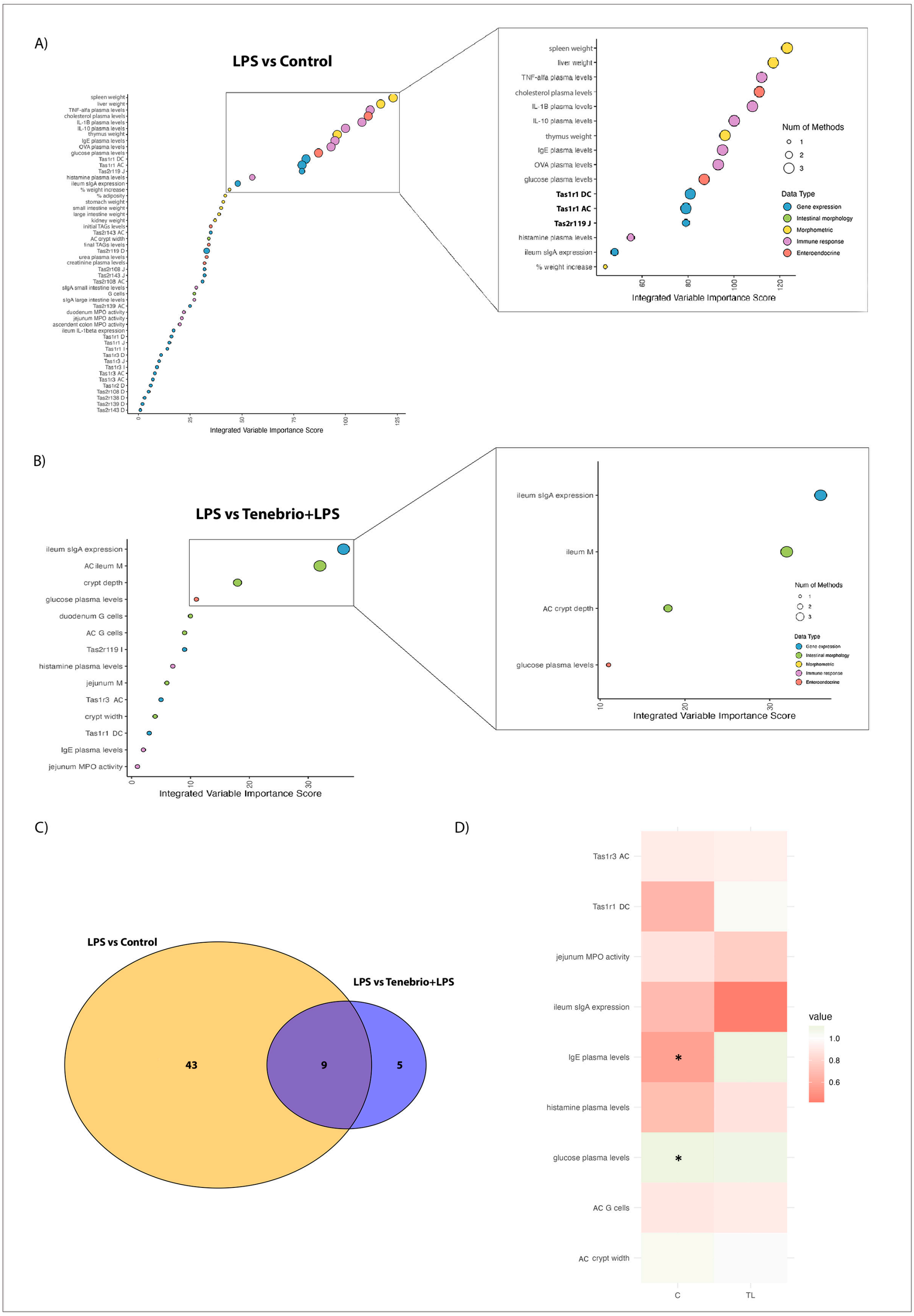

Figure 5. Principal variables distinguishing between the LPS and control or T+LPS groups. A) and B) integrate the analysis of selected variables using machine learning algorithms, ranking them to distinguish between LPS and C or LPS and T+LPS, respectively. C) Venn Diagram derived from the integrative analysis. D) Fold change Heatmap for the nine variables that are common in the two comparisons. Green color indicates the gene expression upregulation or higher levels of the variable, while red signifies downregulation or reduced levels, compared to LPS. * indicates significant differences. Abbreviations utilized include D (duodenum), J (jejunum), I (ileum), AC (ascending colon), DC (descending colon), G (goblet cells) and M (total absorptive area).

### Interplay between Taste Receptors and Immunological and Metabolic Markers

Integrative analysis earlier identified certain taste receptors with an important role in explaining the effects of an insect-enriched diet in both a healthy and an inflammatory scenario. We then conducted Spearman correlation analysis to assess the relationships between biochemical, morphometric or immunological parameters and taste receptor expression. We ran two separate analyses depending on the situation: the Tenebrio and Buffalo groups on the one hand, and the LPS-injected groups on the other. We focused especially on taste receptors in the jejunum, ileum and ascending colon as these were identified as the primary variables by machine learning analysis.

In the homeostatic situation, integrative analysis highlighted Tas1r1 in the ascending colon as an important taste receptor for explaining insect interaction with the organism. Figure 6 shows that the expression of Tas1r1 has a strong negative correlation with the percentage of goblet cells also present in the colon. Tas2r119 in the jejunum also had an important role in the integrative analysis in both insect species. We can see that the expression of Tas2r119 exhibited a negative correlation with plasmatic levels of ovalbumin, a parameter associated with the integrity of the intestinal barrier. Moreover, the expression of the ileal taste receptors demonstrates strong positive correlations with those of ileal secretory IgA: specifically, Tas2r138, -139 and -143 ($p<0.001$).

With regard to the interplay between taste receptor expression and metabolic parameters in this homeostatic condition, plasmatic cholesterol is positively correlated with bitter taste receptors of the jejunum and, in particular, displays a highly significant correlation with Tas2r143 expression. Moreover, this receptor was also positively correlated with GLP-1 and negatively correlated with ghrelin plasmatic levels.

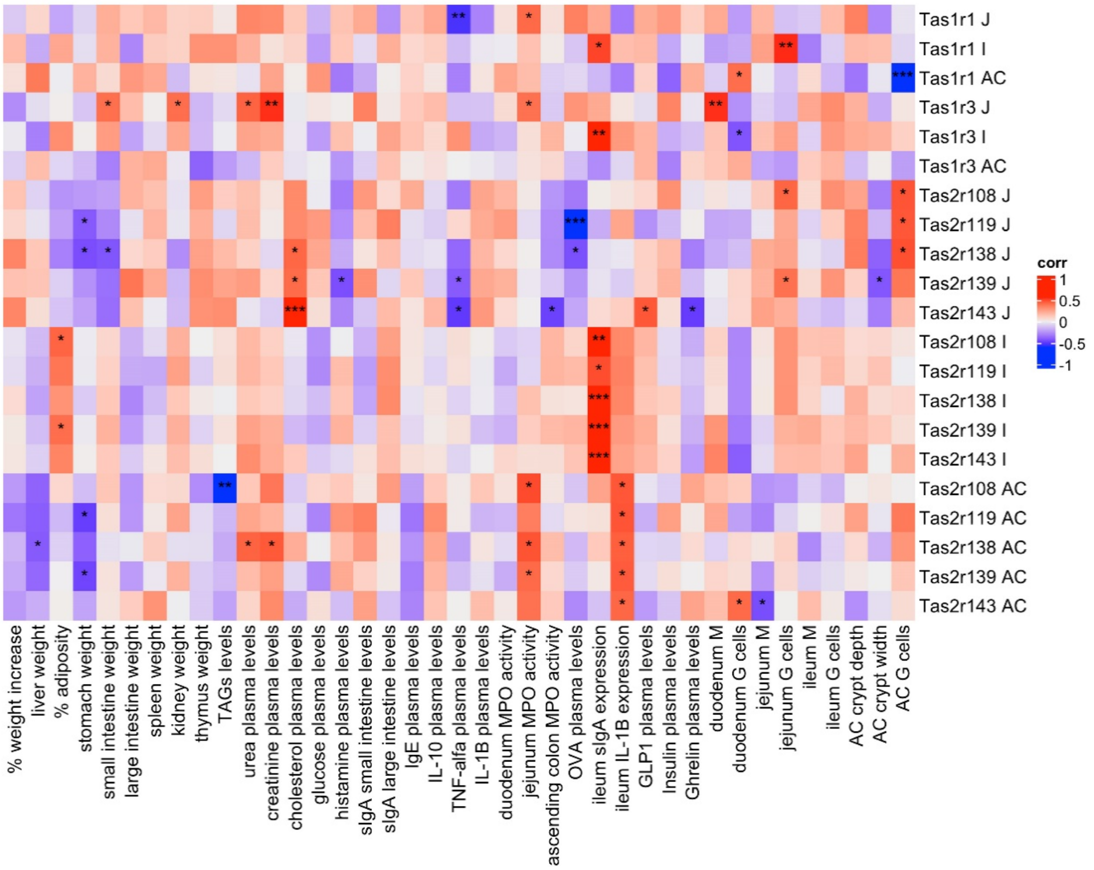

**Figure 6.** Heatmap of spearman correlations between taste receptors relative expression and the other parameters in Tenebrio and Buffalo groups and control. Red color indicates positive correlations while blue color indicates negative correlations. *, ** or *** correlation's p-value <0.05, 0.01 or 0.001, respectively.

In the inflammatory scenario, earlier integrative analysis also highlighted the modulation of Tas1r1 in the ascending colon. Correlation analysis related this positively with several plasma cytokines and histamine and negatively with plasma cholesterol levels (figure 7). Moreover, analysis of the Venn diagram and Fold Change heatmap shows that Tenebrio treatment prevented the LPS effect on Tas1r3 expression levels in the ascending colon. Observed are a strong positive correlation between these Tas1r3 and duodenum MPO activity and a negative correlation with final plasma TAG levels, the parameter that showed the highest number of correlations (all of them negative) with almost all taste receptors analysed in the ascending colon. Finally, jejunal bitter taste receptors presented negative correlations with inflammatory cytokines and the intestinal permeability marker (IL-1$\beta$, TNF-$\alpha$, IL-10, and OVA levels, respectively). Interestingly, these negative correlations were significant when correlated with jejunal Tas2r119 expression. Moreover, jejunal bitter taste receptors exhibited positive correlations with final cholesterol levels, whereas negative correlations were observed between plasmatic levels of triglycerides and bitter taste receptors of the ascending colon. Finally, strong positive correlations were observed between jejunal Tas1r1 expression and creatinine and urea plasmatic levels ($p < 0.001$).

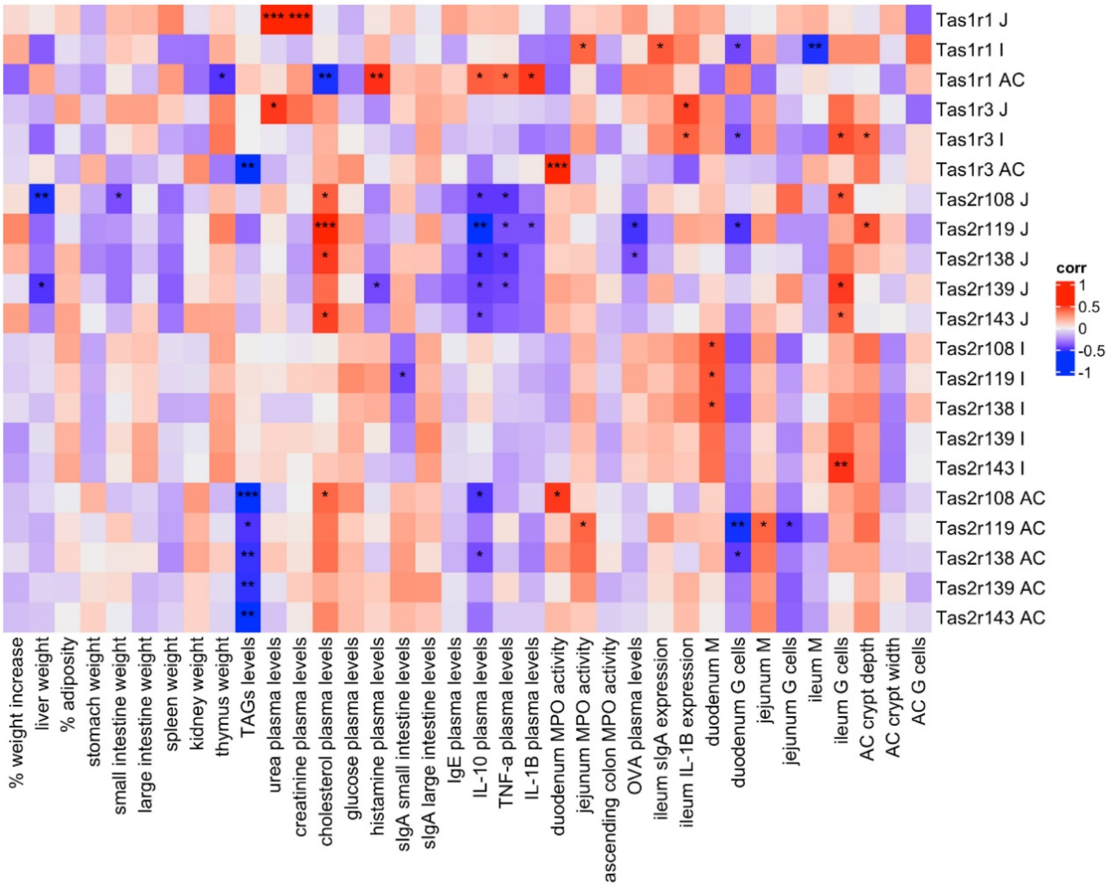

**Figure 7.** Heatmap of spearman correlations between taste receptors relative expression and the other measured variables, in LPS groups and control. Red color indicates positive correlations while blue color indicates negative correlations. *, ** or *** correlation's p-value <0.05, 0.01 or 0.001, respectively.

## Discussion

The present study reveals, for the first time, the modulation of umami and bitter taste receptor expressions with chronic protein supplementation in female rats. The integrative analysis, which considers multiple variables simultaneously and provides a more holistic perspective of the data than single-level analysis [44], identified ascending colonic Tas1r1 expression, along with Tas1r3 and some bitter taste receptors spanning from the jejunum to the colon, as crucial variable distinguishing between the control and the insect-supplemented groups. Additionally, taste receptor expressions significantly correlated with inflammatory and metabolic parameters in both healthy and inflammatory conditions.

Firstly, in a healthy situation, the colonic expression of Tas1r1 and Tas1r3 was upregulated upon supplementation with both Buffalo and *T. molitor*, suggesting taste receptor gene expression can be modulated by insect-derived components in the diet. These findings align with numerous studies in humans and rodents suggesting that chronic exposure to certain dietary compounds, including taste receptor agonists, may lead to changes in mRNA expression of these receptors [45–47]. Umami taste is often associated with the presence of L-amino acids, especially glutamate and aspartate. Insects, known for their high levels of amino acids, including glutamic acid, serve as an excellent source of umami receptor ligands. In fact, glutamate and aspartate are the most abundant amino acids in the raw Buffalo flour used in this study [48]. Regarding amino acid consumption, some authors have shown that dietary glutamate increases the expression of Tas1r1 and Tas1r3 in the stomach and jejunum of piglets, along with other glutamate signalling receptors [49]. Moreover, insect protein is also rich in branched-chain amino acids (isoleucine, leucine, and valine), which have also been described to upregulate umami receptor expression and protein in porcine jejunum [50]. Therefore, considering all these factors, increased umami taste receptor expression may be a response to the levels of amino acids in the diet reaching the gastrointestinal tract.

Interestingly, some authors have shown that, humans and other higher animals, the intestinal secretion of CCK, a satiety hormone, can occurs through the Tas1r1/Tas1r3 activation, while others have also suggested GLP-1 secretion after amino acid activation of this receptor [49,51]. In line with this, previous findings from our research team have demonstrated an increase in plasma GLP-1 levels in rats after the insect supplementation [33]. Moreover, the integrative analysis performed in this study revealed that GLP-1 is another key variable in distinguishing the animal groups that received insect supplementation. Taken together, these results provide more evidence of a possible relationship between umami receptors and hormone secretion in the intestine.

Furthermore, the bitter taste receptors selected by machine learning algorithms were mainly located in the jejunum, the ileum, and the ascending colon. Among these, jejunal Tas2r119 emerged as a key variable that facilitate the differentiation between the insect-supplemented groups and the control group. These results are also consistent with previous research that described the interaction of certain peptides and amino acids with human bitter taste receptors [12], positioning Tas2r119 as particularly susceptible to insect protein consumption. Notably, our findings revealed a significant modulation of both umami and bitter taste receptors in the ascending colon, particularly evident in the colonic Tas1r1 upregulation after insect supplementation and in the Buffalo-supplemented group which showed significantly lower expression levels of Tas2r119 and Tas2r138. These findings may indicate a specific modulation of taste receptors in the colon potentially influenced by the microbiota. The intestinal microbiome has been shown to interact with taste receptors, impacting their expression and function [52,53]. Additionally, research by Borrelli et al. highlights the potential contribution of insects to this modulation [54], as they can serve as sources of short-chain fatty acids (SCFA) known to influence taste receptor expression. SCFA-treated organoids exhibited upregulation of umami gene expression, suggesting a multifaceted mechanism involving not only amino acid composition but also microbial-derived metabolites in taste receptor modulation within the colon [55]. Moreover, recent studies have even reported a microbial-dependent regulation of Tas2r in mice subjected to a long-term high-fat diet [56], further supporting the hypothesis that changes in microbiota composition due to dietary factors may influence taste receptor expression.

The influence of this taste receptor expression modulation on the intestinal function and overall organism responses remains unclear. To gain further insights into the potential implications of the expression changes, we conducted a correlation study between the expression of taste receptors and other evaluated variables. The correlation findings between the expression of colonic Tas1r1 and the percentage of goblet cells in the colon suggested potential interplay with the colonic mucosal environment. Goblet cells, specialized in producing mucus, play a crucial role in maintaining intestinal health and initiating immunological responses [57], reinforcing the idea of Tas1r1 involvement in immune function [58]. Moreover, this connection between goblet cells and Tas1r1 may be produced by the presence of this receptor in colonic tuft cells, which are involved in the immune response that can activate goblet cells [59,60]. However, an additional explanation of this negative correlation could be attributed to the influence of insect compounds on the composition of intestinal cells. Several studies suggested that dietary components like non-digestible carbohydrates and polyphenols can promote L-cell differentiation [61–63]. Therefore, the specific differentiation of those cells expressing Tas1r1, such as L-cells or tuft cells, could logically be related to a decrease in the percentage of goblet cells. Additionally, the positive correlation between the expression of ileal taste receptors and ileal secretory IgA is intriguing and emphasizes the role of taste receptors in the intestinal immune response [64]. IgA acts in the first barrier on mucosa surfaces against infectious microorganisms and toxins. Similarly, antimicrobial peptides, which may be secreted by the stimulation of Tas2r [18], serve as immunomodulators in this context and may impact secretory IgA. These findings collectively set the basis for further research on the specific role of taste receptors in the context of immune response in both healthy and disease situations.

Furthermore, correlation analysis in the two conditions analysed (healthy and inflammatory-induced animals) revealed a negative relation between the mRNA expression of Tas2r119 and intestinal permeability, also suggesting that this receptor is involved in intestinal function and barrier integrity. This association between bitter taste receptors and permeability has been explored in the context of pulmonary endothelium, where bitter taste agonists demonstrated a reduction in the LPS-induced permeability of the pulmonary endothelium in vitro [65].

In this study, we also aimed to analyse the effect of protein supplementation in an inflammatory model. The obtained results indicated that the impact of insect consumption under LPS-induced inflammation were more closely associated with other intestinal parameters, such as inflammatory or morphometry of intestine, rather than with the expression of taste receptors. Despite the non-decisive role of taste receptors in differentiating between insect consumption and control groups under inflammatory conditions, the LPS injection did impact the intestinal expression of taste receptors. This model, which is characterized by the production of a spectrum of cytokines that results in systemic inflammation, particularly manifests an altered physical barrier and a proinflammatory intestinal environment in the small intestine [40,66]. In this inflammatory context, taste receptors may be affected and potentially contribute to the immune response, thus creating a feedback loop [67]. Machine learning algorithms identified eighteen taste receptors that distinguish between the LPS group and the control group. Notably, colonic umami or umami/sweet taste receptors, as well as jejunal Tas2r119, again emerged as the primary contributors to group differentiation. Our univariate analysis showed that the gene expression of Tas2r either did not change or was significantly decreased in certain parts of the intestine of rats with LPS-induced inflammation. Similarly, another study in mice reported an inhibition of taste bud cell renewal after the intraperitoneal injection of LPS [68]. In contrast, a previous study that investigated the expression of some type-II taste receptors in taste buds found that injecting LPS stimulated Tas2rs expression also in mice [69]. Even with these contradictory findings, our results help to establish a connection between taste receptors and immune disturbance, as has previously been described for both Tas1r and Tas2r in humans and rodents [64,70].

Our correlation results (particularly in LPS-injected rats but also in healthy ones) showed negative correlations between plasmatic TNF-α, IL-1β and IL-10 levels and bitter taste receptors (especially Tas2r119) in the jejunum as well as a positive correlation with colonic Tas1r1. Similarly, a study by Reynolds et al. reported an increase in Tas1r1 expression in conjunction with an immune response [22]. Moreover, our study demonstrates that colonic Tas1r3 is also a key discriminator between (i) LPS-injected rats and those in the control group, and (ii) rats injected with LPS alone and those also administered Tenebrio, showing upregulation specifically in the LPS group. This is in line with the results of the study by Shon et al., which suggest that Tas1r3 is a mediator of intestinal inflammation in mice [4].

Finally, interesting correlations are observed in the LPS-treated groups with regard to the biochemical variables analysed. While final triglycerides are negatively correlated with ascending colon taste receptors, cholesterol levels are positively correlated with jejunal bitter ones. In this context, previous studies in humans and rodents have reported a possible relationship between intestinal bitter taste receptors and lipid metabolism [71]. In addition, recent research has added a new dimension to this relationship by showing that cholesterol acts as an agonist for bitter taste receptors, modulating their function [72,73]. Together, these findings highlight the intricate interplay between lipid metabolism and taste receptor signalling pathways, potentially opening up new avenues for understanding and treating metabolic diseases. Furthermore, creatinine and urea plasmatic levels correlate with jejunal umami taste receptors, suggesting possible links between umami taste perception and amino acid metabolism. Dietary glutamate and aspartate are metabolised during intestinal transport to various products, including urea metabolites [74,75]. In this regard, previous findings from our group showed that insect supplementation in rats ameliorates LPS-altered urea levels in plasma [37], reinforcing the link between L-amino acid levels reaching the jejunum and plasma urea levels. Thus, the correlations observed in our study raise the possibility that activation of umami intestinal taste receptors could play a role in the regulation of nitrogen metabolism. All this novel evidence poses questions about the potential systemic effects of taste receptor modulation beyond the digestive system and suggests that taste receptors can be explored as potential targets for treating disturbances.

One limitation of our study is that while we observed modifications in gene expression, these changes may not necessarily translate directly to alterations in protein levels. This is mainly related to the level expression of these receptors, and the scarce availability of technical tools run this screening to quantify it as transmembrane proteins. Additionally, our analysis was conducted on whole intestinal tissue without specific cell-level resolution. However, it is essential to note that our study serves as an initial screening, revealing the modulation of taste receptor expression by different treatments. This preliminary investigation provides a foundation for future research to delve deeper into the most significant receptors and their specific cellular locations within the intestinal tract, as well as to elucidate the underlying mechanisms involved. Further studies focusing on these aspects are crucial for a comprehensive understanding of how insect protein supplementation influences taste receptor expression and function in the gut.

In conclusion, this study demonstrates the modulation of taste receptors after various interventions in rat models. Our data postulate the expression of Tas1r1 in ascending colon as a relevant taste receptor regulated by insect consumption. The consistency of our results across multiple analytical methods strengthens the validity of our findings, which suggest that insect supplementation or induced inflammation modified taste receptor expression, particularly colonic umami taste receptors, that could involve changes in intestinal function and systemic health. Moreover, the intricate network of correlations between taste receptor expression and several physiological parameters, such as various morphological, biochemical, and inflammatory parameters, emphasizes the complexity of interactions within and beyond the gastrointestinal system. Hence, our findings can contribute to a better understanding of the complex mechanisms regulating diet-health interactions, facilitating the development of targeted nutritional interventions towards enhancing intestinal health and overall well-being. Nevertheless, further research is needed to fully elucidate the direct relationship between intestinal taste receptors, gut functions, and overall health.

## Acknowledgment

We would like to thank Harry Park, a PhD Student in Centre for Computational Biology at Duke-NUS Medical School, Singapore, for her assistance in the integrative data analysis. We also thank Niurka Llópiz, Rosa M. Pastor for the respective technical support at the URV. Additionally, our thanks extend to Àngel Ricard Escandell and Sandra Les for their time spent doing laboratory work at the beginning of this project.

## Funding Sources

This research was funded by the Diputació de Tarragona and Universitat Rovira i Virgili (URV) grant number DIPTA-007 2020/26 (ESSARPI), MCIN/ AEI PID2021-122636OB-I00 by ERDF "A way of making Europe", and TED2021-131783B-100 funded by MCIN/AEI/ 10.13039/501100011033 and by the "European Union NextGenerationEU/PRTR". Helena Segú was funded by the Martí i Franquès programme of URV. Florijan Jalševac was funded by Marie Skłodowska Curie grant agreement No 945413 and the Universitat Rovira i Virgili (URV). Mònica Lores was funded by MCIN/AEI/10.13039/501100011033 and FSE+ grant number PRE2022-103004. Montserrat Pinent and Ximena Terra are Serra Húnter fellows.

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# PART 3

Analysis of the chronic consumption of insects as a unique source of protein on intestinal health and general well-being, in healthy and pathological conditions.

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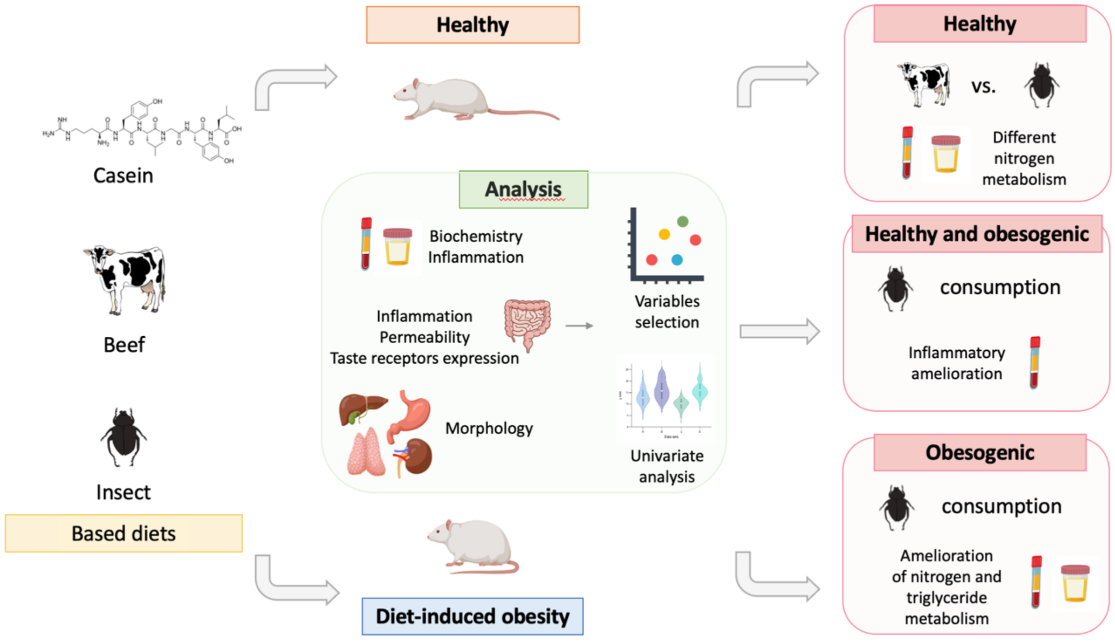
## Manuscript 3

Examining the Impact of Insect-Based Diets on Intestinal and Overall Health, in Healthy and Diet-Induced Obese Rats



# Examining the Impact of Insect-Based Diets on Intestinal and Overall Health, in Healthy and Diet-Induced Obese Rats

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## Abstract

Insects have been proposed as a promising alternative protein source, meeting essential amino acid requirements, and offering environmental advantages over conventional sources. They also contain bioactive compounds that could exert health benefits. Thus, the present study aimed to investigate the effects of chronic consumption of insect-based diets in female rats compared to traditional protein sources, both in healthy and obesogenic conditions. To achieve this objective, female Wistar rats were randomly assigned to one of five groups. Each group was fed a different diet for a period of four weeks. Then the groups were divided as follows: a casein-based diet (Control, n=8), a beef-based diet (Beef, n=8), or an insect-based diet (Insect, n=8), in the healthy condition. Furthermore, in the obesogenic condition, the rats were subjected to a 12-week cafeteria diet, as well as to the control diet (CAF, n=8) or the insect diet (CAF-I, n=8). We employed an integrative analytical approach to identify the most significant variables that differentiate between the groups under investigation. These variables included metabolic, inflammatory, morphologic, and intestinal taste receptors parameters. The results revealed that the consumption of insects as a unique source of protein is safe and may confer potential health benefits. The consumption of insect-based diet result in a different nitrogen and lipid metabolism, and lower proinflammatory profile than the consumption of other diets, in health and obesogenic status. Furthermore, the insect consumption can mitigate the alterations in urea and lipids induced by the cafeteria diet, thereby highlighting their potential as a viable alternative protein source.

Keywords: insect-based diet, protein, healthy, obesogenic, intestine.

## Introduction

Insects have emerged as high-quality protein source, that met the requirements of essential amino acids content, and contain unsaturated fatty acids and micronutrients [1,2]. Moreover, the quantity and quality of protein is comparable to the conventional animal- and plant-based protein-dense foods, such as beef, eggs, or soy [3]. Their production requires less land and water use, emits lower greenhouse gas emissions, while exhibit high feed conversion efficiency, making them a cost-effective and sustainable alternative to traditional livestock [4]. Therefore, insects represent a promising sustainable alternative protein source to help meet the food supply challenge posed by population growth, providing a rich source of essential nutrients while mitigating the environmental impacts associated with traditional animal-based protein production [5].

In addition to being consumed as food, insects have been described as containing bioactive compounds that exert health benefits. These include antioxidant, antihypertensive, antimicrobial activity, and immunomodulatory effects [6]. While some studies have investigated the health effects of incorporating insect protein into animal diets, research into the complete replacement of protein intake with insect sources remains limited. Furthermore, few studies have directly compared the effects of insect consumption as a replacement for conventional meat intake in vivo, with most focusing on amino acid absorption and muscle protein synthesis [7,8]. On the other hand, some studies investigating the partial replacement of conventional protein sources with insects, indicated changes in the gut and systemic metabolism. In addition, partial replacement of meat with insect protein in healthy rats showed changes in the intestinal microbiome and endogenous metabolism, likely due to insect protein residues reaching the colon [3]. One study also reported improvements in metabolic health, including reduced lipid levels, in obese Zucker rats when casein was replaced with protein-rich insect powder [9]. In our previous study in rats, which consisted of a low-dose of insect supplementation in both healthy and LPS-induced inflammatory conditions, we observed significant improvements in various parameters affected by LPS-induced disturbances, promoting overall health and alleviating some of the associated disruptions [10]. Thus, the present article would primarily assess the impact of insect as a unique source of protein in both healthy and pathological situations.

Western dietary patterns, characterized by high consumption of dairy products, meat, and eggs; often exceed recommended dietary thresholds for sugars, saturated fats, and cholesterol [11], contributing to chronic inflammatory diseases, such as obesity, diabetes, and some gastrointestinal diseases as inflammatory bowel disease [12]. These diets have been related to an increased intestinal permeability, intestinal dysbiosis and disturbed inflammatory and metabolic response. Dietary and lifestyle changes are the major recommendations to mitigate or reverse these effects [13], with growing interest in functional foods and bioactive ingredients with anti-inflammatory and weight-loss properties, or some other beneficial effects such as the improvement of gut microbiota composition [14]. In this context, insects have been postulated as a promising protein source of protein rich in bioactive compounds, offering potential application in the prevention and management of chronic diseases [15]. In this sense, a study involving obese mice demonstrated a reduction in body weight and metabolic amelioration when consumed mealworm-based diet, suggesting that the consumption of mealworms could confer health benefits to obese patients [16].

Although some studies have indicated potential benefits of insect consumption on metabolic, inflammatory and intestinal health, further research is required in both healthy and pathological conditions. This should involve the use of different insect species and the analysis of a wider range of health parameters [17–19]. Therefore, the main objective of the present study was to analyse the effects of insect-based diet on general health and, particularly, in the intestinal tract in both healthy and pathological conditions, comparing them with casein and beef-based diet. The evaluated morphometric data, biochemical and inflammatory plasma parameters, and intestinal inflammatory and taste receptor expression were analysed using machine learning algorithms to identify the most significant variables affected by each diet.

## Materials and methods

### Diets

The diets were prepared by IRTA. in such a way that the nutritional composition was approximately the same in all of them (supplementary table 1). In this sense, casein, beef or insect powder were used to prepare each diet and obtain the same amount of protein. The other nutrients were balanced to ensure that the diets were nutritionally comparable.

Cafeteria (CAF) diet included milk with sugar, sausages, carrot, bacon, biscuits with pâté, and muffins ad libitum, which induces hyperphagia.

### Experimental design

A total of 40 six-week-old RccHan: WIST female rats (Janvier, Castellar del Vallès, Spain) spent an adaptation period of 14 days in the Universitat Rovira Virgili animal facility under standard conditions. They were caged in pairs at 22 °C with a standard 12-hour light-dark cycle, ventilation, ad libitum access to tap water and a standard Teklad diet (Cat No: Teklad 2014, Envigo++, Barcelona, Spain).

After this acclimatation period, they were individualized and randomly distributed into five experimental groups (n=8). Of these groups, three represented healthy conditions, while the remaining group represented pathological conditions, as described below:

Healthy condition:

- Control group: fed a diet in which the protein content was casein.

- Beef group: fed a diet in which the protein content was obtained from beef powder.
- Insect group: fed a diet in which the protein content was obtained from insect (Alphitobius diaperinus) powder.

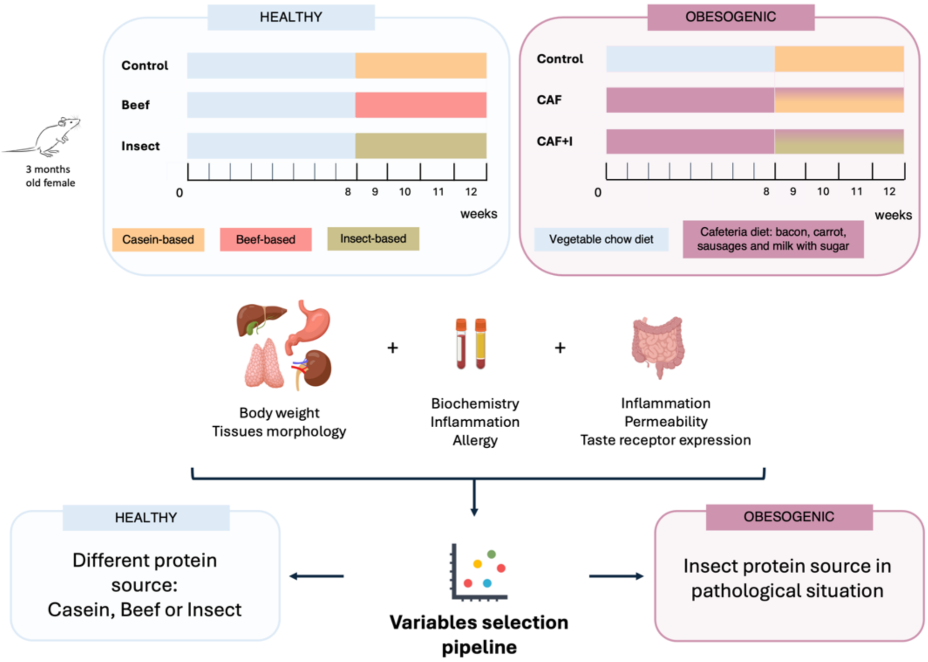
Obesogenic condition:

- Group CAF: consumed cafeteria diet plus the control diet.
- Group CAF-I: consumed cafeteria diet plus insect-based diet.

In this condition, the control group was used as a control in the subsequent data analysis.

During 8- weeks, CAF and CAF-I groups were fed with cafeteria diet, while the other three groups received a standard chow diet. After this period, each group was transitioned to its respective specific protein-based diet for four weeks. The CAF group continued on the cafeteria diet, while the CAF-I group received both the cafeteria diet and the insect-based diet. The experimental design is shown in Figure 1.

**Figure 1**. Experimental design.



During the study, body weight was measured once a week, and the health status of the animals was regularly monitored for cleanliness, general physical appearance, faecal consistency, and stress symptoms. The food intake was also measured daily when the dark cycle finished, and the active phase of the animal began. In the last week of experiment, urine sampling was performed, and intestinal permeability test was carried out (oral given ovalbumin).

At the end of the experiment, the animals were euthanised by decapitation. Some of main organs (right kidney, liver, spleen, thymus, brown adipose tissue, small and large intestine) were excised, weighed, and immediately frozen in liquid nitrogen. White abdominal adipose tissue (WAT) from the mesenteric, retroperitoneal and epididymal locations was excised and weighed to calculate the percentage of adiposity. Prior to intestinal processing, intestinal and colonic lavage fluids were collected with approximately 4ml of PBS, and frozen at -80°C for analysis of the mucosal inflammatory response. Fat was then removed, and according to the intestinal segment described by Vdoviaková et al. 2015 [17], samples from the duodenum, jejunum, ileum, ascending colon, and descending colon were demarcated. In the jejunum, ileum, and ascending colon, the relative gene expression of some selected taste receptors (tas1r and tas2r), and inflammation-related genes was analysed.

Blood was collected with EDTA as anticoagulant. Plasma was obtained by centrifugation at 1500 g for 15 min at 4°C and frozen immediately at −80 °C for future parameter quantification. All procedures were approved by the GENCAT Animal Experimentation Committee (number 11817). The biochemical, inflammatory, and allergenic markers were analysed in plasmatic samples.

### Biochemical analysis

Colorimetric kits from QCA (Tarragona, Spain), Materlab (Madrid, Spain) were used to determine the following plasma biochemical parameters: total cholesterol (QCA, Ref 995282), glucose (QCA, Ref. 998282), triacylglycerols (TAGs) (QCA, Ref 992330); as well as plasmatic and urine levels of urea (QCA, Ref.993648). Non-esterified fatty acid (NEFA) levels were also assessed by colorimetry (Wako, Ref. 270-77000). Commercial ELISA kit was used to quantify plasma levels of insulin (Millipore, Madrid, Spain).

### Analyses of intestinal barrier and immune response including allergy

The ovalbumin (OVA) test was used to determine intestinal permeability [18]. These analyses were carried out using Ovalbumin ELISA kits (MBS2000240) from MyBioSource (San Diego, CA, USA).

Plasma levels of total IgE and histamine were measured as biomarkers of allergy, while plasma levels of IL-10, TNF-α and IL-1β were determined as inflammatory cytokine markers. All of them were analysed by ELISA kits from Elabscience (Texas, United States): IgE (E-EL-R0517), histamine (E-EL-0032), IL-10 (E-EL-R0016), TNF- α (E-EL-R2856), IL-1β (E-ELL-R0012) and performed according to the manufacturer's instructions.

Secretory IgA (sIgA) levels were measured in small and large intestinal lavage fluids using the ELISA kit from MyBioSource (MBS9711882).

## Intestinal gene expression

Total RNA and cDNA were obtained as previously reported [19]. Quantitative PCR amplification was performed using specific TaqMan® probes for the sIgA inducing protein (Rn01406210_s1), IL-1β (Rn00580432_m1), Tas1r1 (Rn01516038_m1), Tas1r3 (Rn00590759_g1), Tas2r108 (Rn02396427_s1), Tas2r119 (Rn00576950_s1), Tas2r138 (Rn04218919_s1) and PPIA (cyclophilin) (Rn00690933_m1) genes. The relative expression of each gene was compared with the control group using the 2-ΔΔCt method and with the PPIA gene as a reference.

## Statistical analysis

### Integrative Analysis and Variable Selection

An integrative analysis of the forty-seven analysed variables was carried out. Machine learning algorithms (Elastic Net, Partial Least Squares Discriminant Analysis (PLS-DA), and Random Forest (RF)) were used to generate a set of scores that reflected the importance of variables in distinguishing between groups, as previously described (article accepted to be published in Journal of Agricultural and Food Chemistry).

As mentioned before, our experimental design involves two different scenarios: one with healthy female rats, including Control, Beef and Insect; and the other with rats under obesogenic conditions, including the cafeteria groups (CAF, CAF-I). Consequently, we performed two separate analyses, and the algorithms were applied in consideration of these two scenarios: (1) comparison of Control, Beef, and Insect, and (2) comparison of Control and CAF+I with CAF. Therefore, this approach allowed us to examine the effects of insect consumption in (1) a healthy scenario, and in (2) a diet-induced obesogenic status.

A Venn diagram was then created integrating the results of the comparisons in each scenario. The results of the Venn diagram in the obesogenic status were visualised by a fold change heat map, which describes how much the variable changes between two conditions as well as the direction of the change. The fold change of selected variables was calculated as A/B, where A is the mean of the CAF or CAF-I variable and B is the mean of the control or CAF, respectively.

### Univariate analysis

A normality test was conducted for each group using the Shapiro-Wilk test. Univariate statistical differences were assessed by Tukey's multiple comparison test between the Control, Beef and Insect groups; or Mann-Whitney U test between the Control, CAF and CAF-I groups. Data are presented as mean ± SEM and were analysed with the XLSTAT 2023 statistical software (Addinsoft, USA). Mean differences were considered statistically significant if $p < 0.05$.

## Results

### Healthy inflammatory, metabolic and intestinal profile of insect consumption as a unique source of protein in a healthy situation

We used machine learning algorithms to integrate and identify, among the forty-seven variables analysed, the key variables that most effectively discriminated between the groups according to the of the protein intake (casein as control, beef or insect). The analysis was performed by pairing the groups.

Elastic Net, PLS-DA and Random Forest analyses identified eight key variables that discriminated between control and insect groups. These variables were then integrated, ranked, and visually presented in the plot shown in Figure 2A. In this sense, we could see that the most important variables (the ones selected by more than one method) for discriminate between casein and insect consumption were principally immune or allergenic related parameters, and taste receptor gene expression. On the other hand, ten variables discriminated between control and beef groups which included not only immune related parameters and taste receptor gene expression, but also metabolic-related variables (Figure 2B). Furthermore, the integrative analysis of beef and insect comparison identified a greater number of variables, twenty-six in total. Once again, allergenic, immunological and metabolic variables were identified among the most important. However, the algorithms also selected two morphometric variables that were related to metabolic status: the percentage of adipose tissue and the percentage of body weight gain (Figure 2C).

In order to integrate all this information, we performed a Venn diagram (Figure 2D) which allowed us to focus on the variables selected by more than one method and in more than one comparison based on protein source.

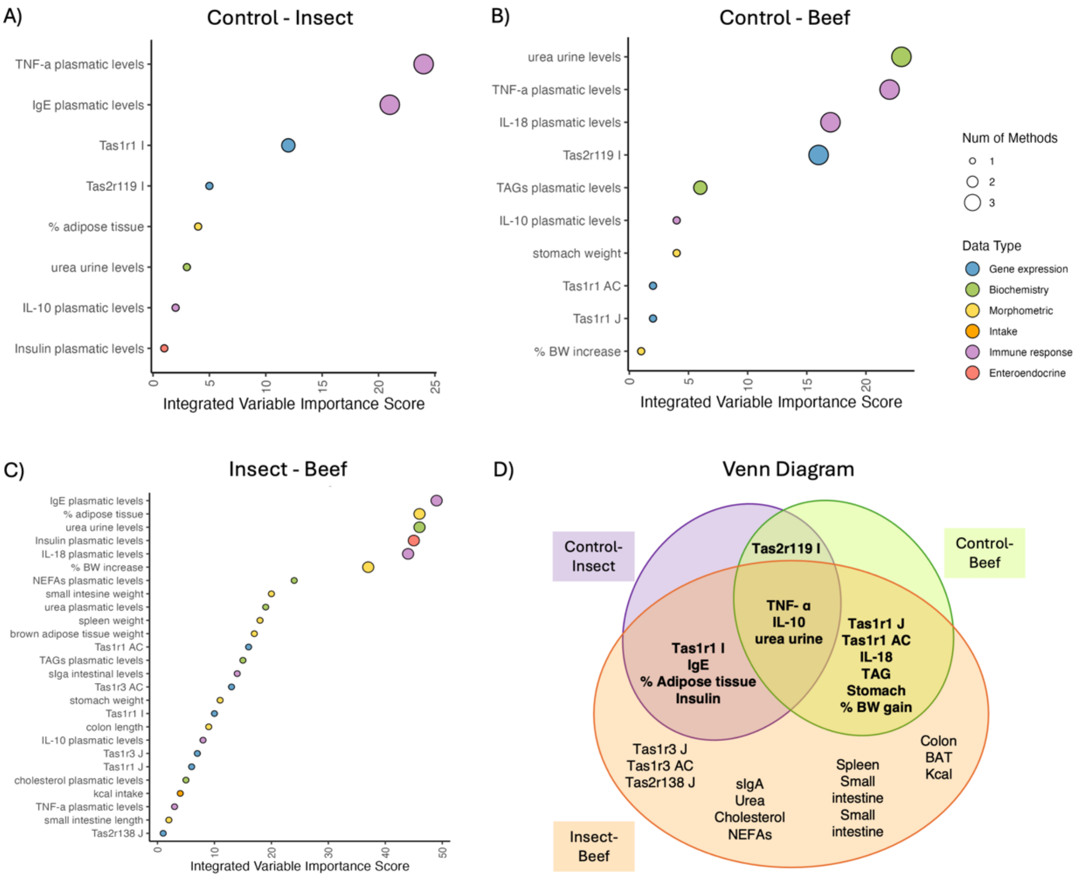


**Figure 2.** Principal variables distinguishing between the Control, Beef and Insect groups. Graphs integrate the analysis of selected variables using machine learning algorithms, ranking them to distinguish between A) Control and Insect groups, B) Control and Beef groups, and C) Insect and Beef groups. D) Represents Venn Diagram that integrate A), B) and C) graphs; where each ellipse represents each comparison made, and the overlaps between ellipses include the variables selected in more than one comparison. Bold type variables are the ones selected in more than one comparison. Abbreviations were used to describe: J, jejunum; I, ileum; AC, ascending colon; OVA, ovalbumin; BW, body weight.

Among the metabolic parameters, urinary urea levels were selected for the three comparisons. Univariate analysis showed that urinary urea decreased in rats consuming the beef-based diet compared to the control group (Figure 3A). Given the importance of this variable we also compared plasma urea levels between groups (although it was not a selected as a key variable) and calculated the ratio of urine to plasma levels to obtain more information about this change. Plasma urea levels decreased in the insect group compared to the beef group (Figure 3B), while the ratio of urine to plasma levels was significantly lower in the beef group compared to the control (Figure 3C). As can be seen in the Venn diagram, other metabolic variables that were important in distinguishing between groups were triglycerides and plasma insulin. However, in this case they were only selected in two of the comparisons. Univariate analysis showed that triglycerides were increased in beef compared to the control group (Figure 3D). On the other hand, insulin did not change between groups, as was the case for the other biochemical parameters (Supplementary Table 2).

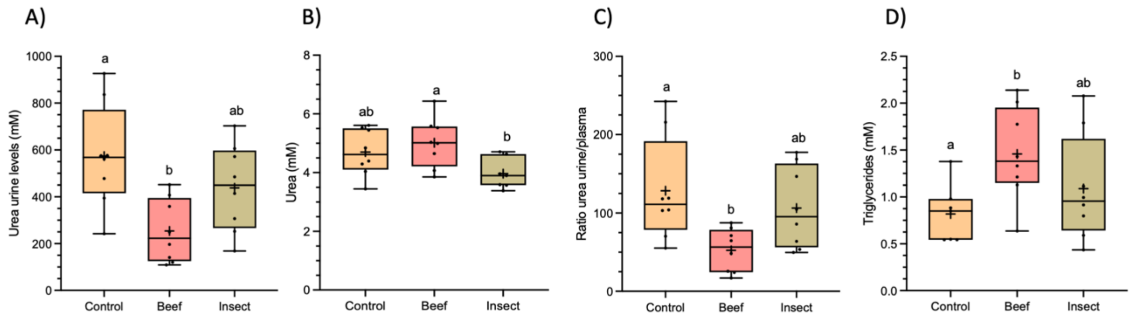


**Figure 3.** Biochemical and metabolic variables selected in integrative analysis. The data are presented in a box and whiskers plot. Boxes represent the median and interquartile range, while whiskers extend from the smallest to the largest values, encapsulating the full range of the data (n=7/8). Different letters indicate differences using Tukey's multiple comparison test ($p < 0.05$).

The selected plasmatic immune and allergenic variables included TNF-a, IgE, IL-18, and IL-10. However, only TNF-α plasmatic levels were found to be significantly decreased in animals consuming the insect diet compared to the control (Figure 4A). No significant differences were observed in the other inflammatory variables analysed (Figure 4B-D and Supplementary Table 3).

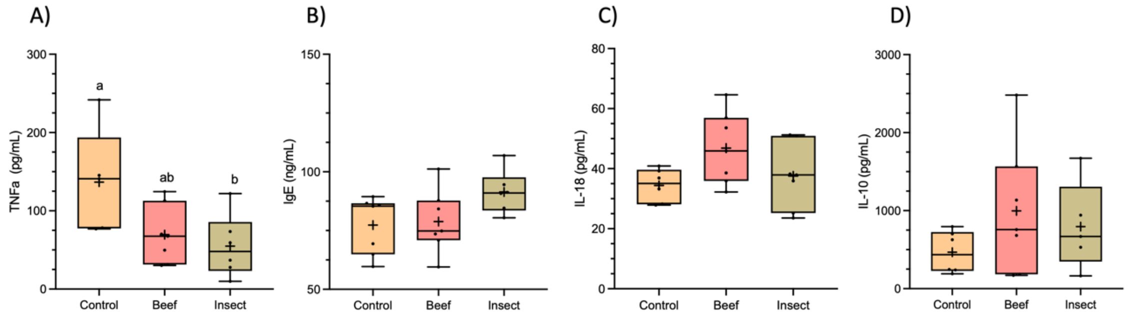


**Figure 4.** Inflammatory and allergenic variables selected in integrative analysis. The data are presented in a box and whiskers plot. Boxes represent the median and interquartile range, while whiskers extend from the smallest to the largest values, encapsulating the full range of the data (n=7/8). Different letters indicate differences using Tukey's multiple comparison test ($p < 0.05$).

The paired integrative analysis also selected some morphometric variables. The percentage of adipose tissue and body weight and stomach weight were the main variables selected by machine learning algorithms in more than one comparison. However, in the univariate analysis, none of these variables showed significant differences between groups (Table 1), as was the case for the other morphometric parameters (Supplementary Table 4).

**Table 1**. Morphological parameters analysed in Control, Beef and Insect groups. Results are presented as the mean ± SEM (n=7/8).

| | Control | Beef | Insect |
|---|---|---|---|
| Body weight gain (%) | 23.4 ± 2.91 | 26.7 ± 2.65 | 21.9 ± 3.31 |
| Adipose tissue (%) | 5.80 ± 0.39 | 5.91 ± 0.47 | 4.87 ± 0.24 |
| Stomach weight (g) | 1.20 ± 0.02 | 1.26 ± 0.04 | 1.21 ± 0.04 |

Finally, regarding the expression of taste receptor genes, ileal tas1r1 and tas2r119, as well as the relative expression of colonic and jejunal tas1r1, were the most important selected parameters discriminating between healthy rats. In this case, only tas2r119 expression in the ileum showed a significant downregulation in the beef group compared to the control group (Figure 5A). Although selected by machine learning in more than one comparison, tas1r1 gene expression in different locations did not show significant changes between groups (Figure 5B-D). The other tas1r and tas2r expressions assessed also did not change (Supplementary Figure 1).

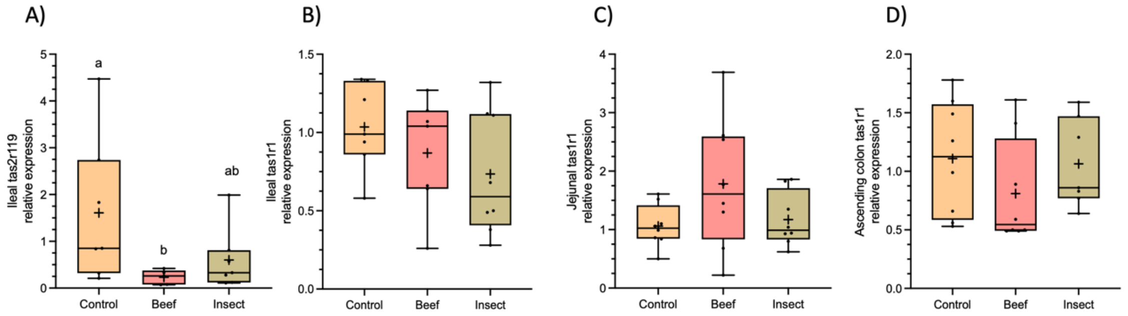


**Figure 5**. Taste receptor expression that was selected by integrative analysis. The data are presented in a box and whiskers plot. Boxes represent the median and interquartile range, while whiskers extend from the smallest to the largest values, encapsulating the full range of the data (n=7/8). Different letters indicate differences using Tukey's multiple comparison test ($p < 0.05$).

## Protective immunometabolic effects of the consumption of insects in a diet-induced obesogenic state

From the total of forty-seven variables analysed in the obesogenic condition induced by the cafeteria diet, we again selected those that better differed between groups using machine learning algorithms. In this case, the CAF group was compared with the control group to see which variables were more susceptible to change by the cafeteria diet, and with the CAF-I group to observe the effect of insect consumption in this pathological condition.

According to the integrative analysis (Figure 6A), twenty parameters were important to indicate the presence of the pathological situation induced by 12 weeks of cafeteria consumption in rats. As expected, the most important variables were total caloric intake, morphometric variables related with adiposity and body weight, insulin secretion and inflammatory variables.

When comparing the groups of rats the consumed cafeteria diet with those that also received an insect-based diet, a total of twenty-two variables discriminated between insect and non-insect consumption (figure 6B). Among several immune, morphometric and biochemical parameters, the relative expression of intestinal taste receptors also gained importance for indicate the insect consumption.

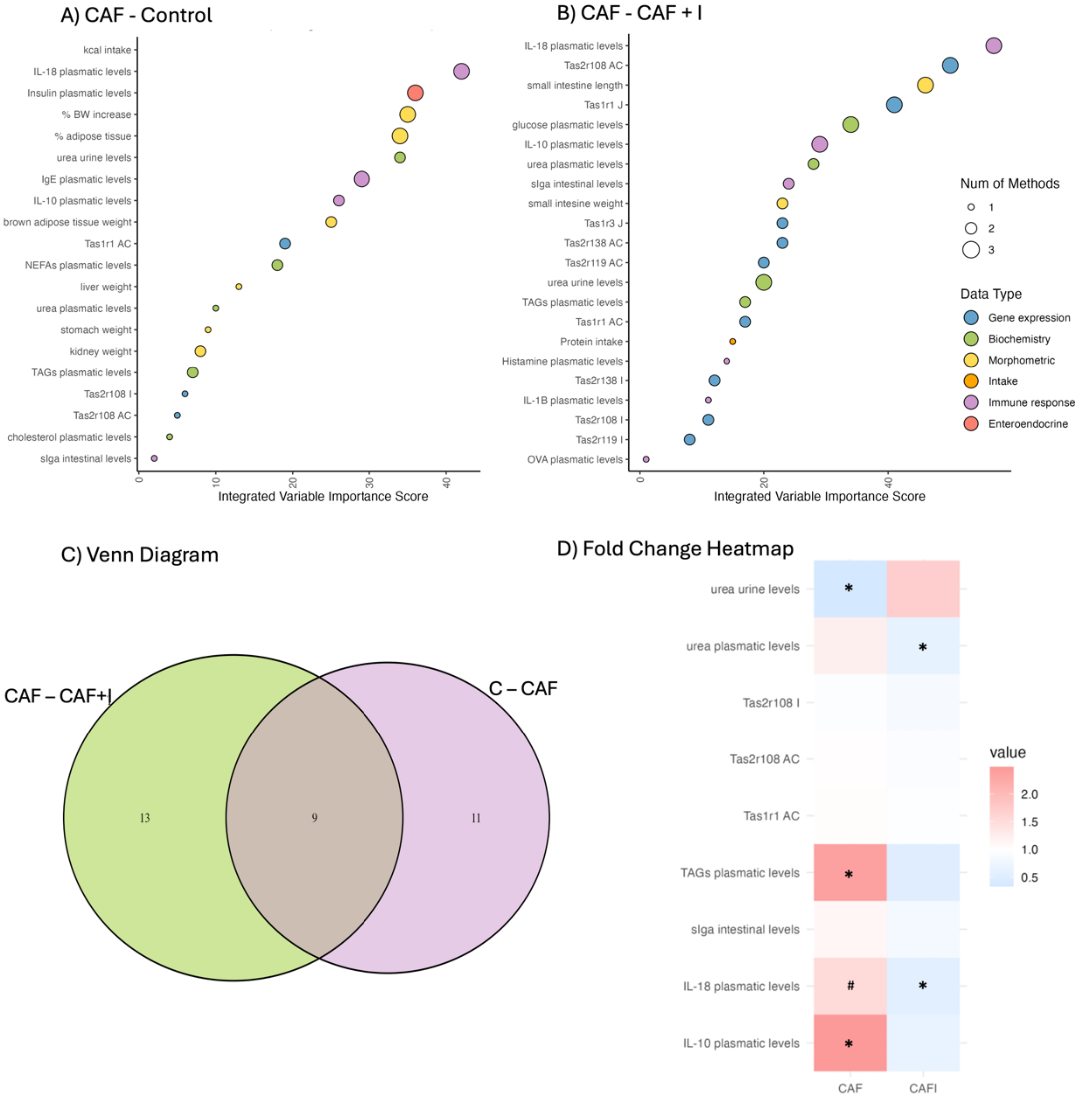


**Figure 6**. Principal variables distinguishing between the CAF group and Control or CAF+I. A) and B) integrate the analysis of selected variables using machine learning algorithms, ranking them to distinguish between CAF and Control or CAF+I, respectively. Abbreviations were used to describe: J, jejunum; I, ileum; AC, ascending colon; OVA, ovalbumin; BW, body weight. C) Venn Diagram derived from the integrative analysis. D) Fold change Heatmap for the thirteen variables that are common in the two comparisons. Red color indicates the gene expression upregulation or higher levels of the variable, while blue signifies downregulation or reduced levels, compared to CAF. * indicates p-value < 0.05, # indicates p-value < 0.1.

Interestingly, by integrating these two comparisons in a Venn diagram (Figure 6C), we obtained that nine variables were modified not only by the cafeteria diet, but also by the insect consumption in this diet-induced obesogenic situation. By observing these variables and their corresponding variation between groups, we were able to determine whether the consumption of an insect-based diet could exert a possible compensatory effect on the changes induced by the cafeteria diet (Figure 6D). In this sense, the levels of urea and plasma triglycerides were significantly altered by the cafeteria diet but reversed by the insect-based diet consumption. The same was observed for IL-10 and IL-18, the latter tending to increase in the CAF group and statistically decreasing with insect consumption.

To further investigate the effect of an insect-based diet in obesogenic conditions, we also analysed the univariate difference on the main variables selected by two or three methods of machine learning algorithms. In this sense, kilocalorie intake was the main variable that differed between the CAF and control groups. As expected, it increased when the animals consumed the cafeteria compared to the control group, regardless of whether the animals consumed insect protein or not (Figure 7).

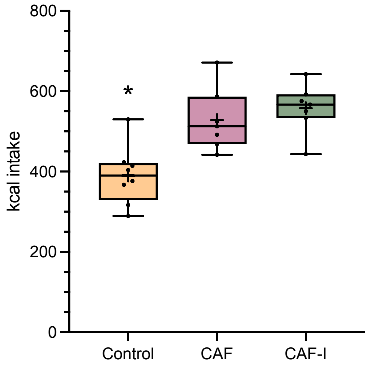


**Figure 7.** Kilocalories intake of control, CAF and CAF + I groups. The data are presented in a box and whiskers plot. Boxes represent the median and interquartile range, while whiskers extend from the smallest to the largest values, encapsulating the full range of the data (n=7/8) * indicates statistical difference with respect to CAF group, using Mann-Whitney U test ($p < 0.05$).

These could be explained by the increased consumption of fats in the cafeteria groups compared with the control group, to the detriment of fibre intake, while they consumed the same amount of carbohydrate and protein (Supplementary Figure 2). Furthermore, examination of individual food consumption within the cafeteria-fed groups showed that they obtained more kilocalories from milk than from feed, and more from feed than from bacon, carrots and sausages, with no differences between the CAF and CAF-I groups (Supplementary Figure 3).

At the biochemical level, TAGs plasmatic levels were significantly higher in cafeteria rats (Figure 8A). Plasma urea levels were significantly lower in insect-fed rats compared to the cafeteria group (Figure 8B), while urinary urea levels were lower in cafeteria rats compared to both control and CAF-I groups (Figure 8C). In addition, the urine/plasma urea ratio analysed was significantly lower in the CAF group compared to the control but also to CAF-I, indicating a return to the control state with insect consumption (Figure 8D). Plasma levels of NEFA's, cholesterol and insulin did not change between groups (Supplementary Table 5).

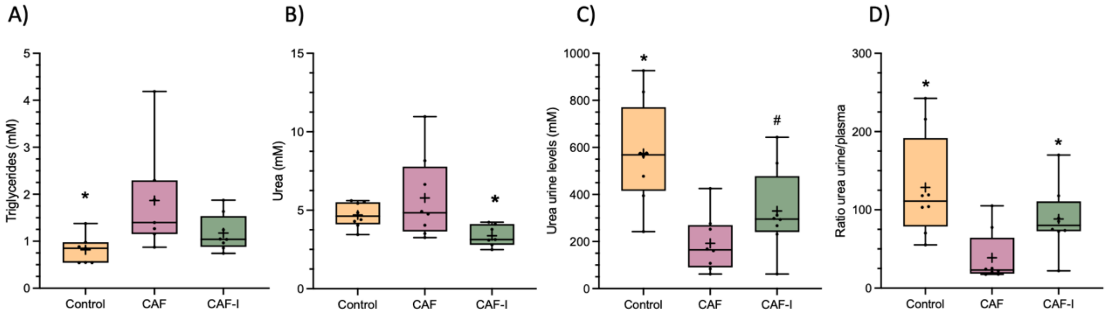


**Figure 8.** Biochemical variables selected in the integrative analysis The data are presented in a box and whiskers plot. Boxes represent the median and interquartile range, while whiskers extend from the smallest to the largest values, encapsulating the full range of the data (n=7/8). # indicates trend and * indicates statistical difference compared to CAF group using Mann-Whitney U test (0.1>p<0.05; and p < 0.05 respectively).

According to the immune response, IL-18 levels tended to increase in the cafeteria group compared to the control, whereas the CAF+I group significantly reduced plasmatic levels compared to the cafeteria and returned to homeostatic levels (Figure 9A). IL-10 was also increased by cafeteria consumption compared to control, with no effect of insect consumption (Figure 9B). On the other hand, although intestinal secretory IgA and plasmatic IgE levels were also selected by machine learning, no significant changes were observed (Figure 9C-D). Furthermore, TNF-a, IL-1β, histamine and OVA plasmatic levels and ileal sIgA and IL-1β gene expression did not differ between groups (Supplementary Table 6).

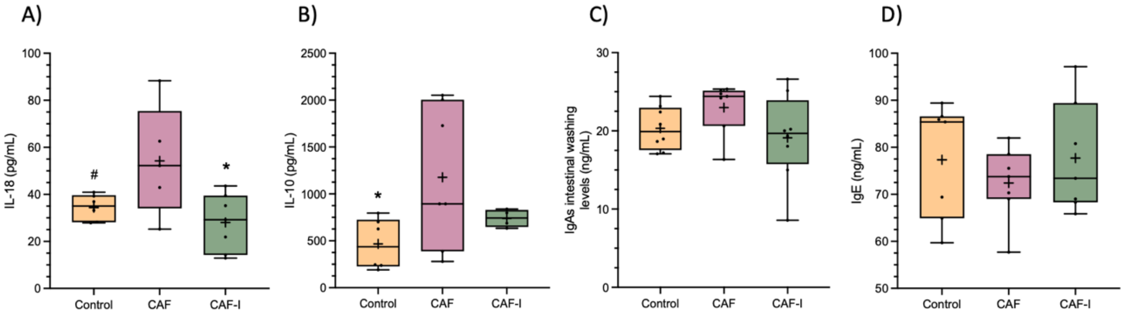


**Figure 9.** Inflammatory and permeability parameters selected in the integrative analysis. The data are presented in a box and whiskers plot. Boxes represent the median and interquartile range, while whiskers extend from the smallest to the largest values, encapsulating the full range of the data (n=7/8). # indicates trend and * indicates statistical difference compared to CAF group using Mann-Whitney U test (0.1>p<0.05; and p < 0.05 respectively).

As expected, some morphological variables were altered when rats consumed cafeteria diet. Statistical analysis showed that these rats showed an increase in percentage body weight gain, fat and brown adipose tissue, stomach and liver weights, and a trend towards increased kidney and small intestine weights. On the other hand, CAF+I group resulted in a more length of the small intestine when compared to CAF (Table 2). In contrast, spleen, caecum and colon weight and colon length did not differ between groups (Supplementary Table 7).

**Table 2.** Morphological parameters selected in the integrative analysis. Results are presented as the mean ± SEM (n=7/8). # indicates tendency and * indicates statistical difference observed when compared to CAF group using Mann-Whitney (0.1>p<0.05; and p < 0.05 respectively).

| | Control | CAF | CAF - I |
|---|---|---|---|
| Body weight gain (%) | 23.4 ± 2.91 * | 51.0 ± 8.47 | 48.2 ± 4.77 |
| Adipose tissue (%) | 5.80 ± 0.39 * | 8.80 ± 0.78 | 9.05 ± 0.61 |
| Brown adipose tissue (g) | 0.56 ± 0.05 * | 0.91 ± 0.08 | 0.92 ± 0.06 |
| Liver weight (g) | 8.01 ± 0.46 * | 10.0 ± 0.50 | 9.50 ± 0.44 |
| Kidney weight (g) | 0.80 ± 0.03 # | 0.93 ± 0.04 | 0.98 ± 0.04 |
| Stomach weight (g) | 1.20 ± 0.02 * | 1.33 ± 0.05 | 1.28 ± 0.02 |
| Small intestine weight (g) | 6.61 ± 0.25 # | 7.53 ± 0.32 | 8.20 ± 0.40 |
| Small intestine length (cm) | 101 ± 1.86 | 98.9 ± 1.64 | 106 ± 2.07 * |

Finally, several taste receptors were selected as important variables, especially in animals that consumed the insect-based diet in addition to the cafeteria diet. In particular, taste receptors gene expression in the jejunum and ascending colon appeared to be more sensitive to insect consumption in an obesogenic state. Ascending colon tas1r1 expression was selected by two comparisons, but only the cafeteria diet tended to downregulate its expression compared to the control (Figure 10A). Although colonic tas2r108 was also selected by two comparisons, it was not significantly changed by either cafeteria or insect consumption (Figure 10B). Furthermore, insect consumption reduced tas1r1 and tas1r3 jejunal expression in the pathological situation (Figure 10C-D). Jejunal, ileal and colonic tas2r expression did not show significant differences between groups in the pathological situation, although some of them were selected by machine learning algorithms (Supplementary Figure 4).

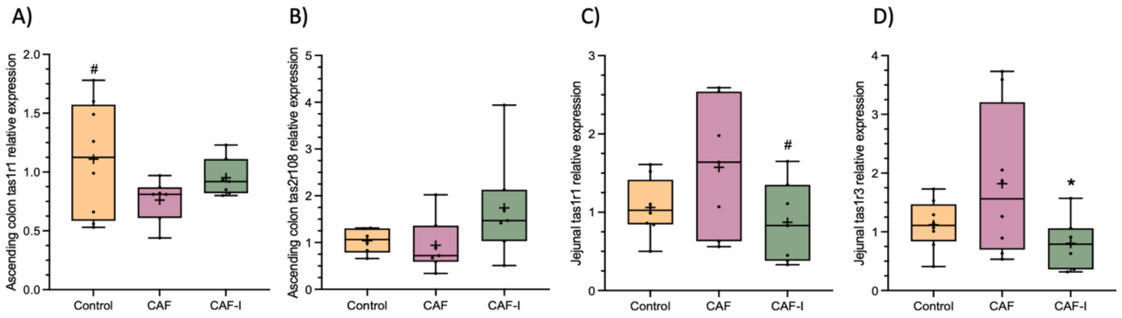


**Figure 10.** Taste receptor expression of genes selected in the integrative analysis. The data are presented in a box and whiskers plot. Boxes represent the median and interquartile range, while whiskers extend from the smallest to the largest values, encapsulating the full range of the data (n=7/8). # indicates trend and * indicates statistical difference compared to CAF group using Mann-Whitney U test (0.1>p<0.05; and p < 0.05 respectively).

## Discussion

In a context where there is a need to find new sources of dietary protein as part of the great food transformation to support environmental sustainability while promoting human health, the impact of insect consumption on health is an emerging field of study [20]. In this sense, the present study reveals, for the first time, the overall effects of chronic consumption of an insect-based diet in female rats, compared to casein or beef-based diets under healthy condition and in the context of a cafeteria diet-induced pathological situation. By examining several physiological parameters, our results demonstrated the relationship between dietary composition and physiological responses. In particular, in the context of inflammation and metabolism, our study showed health benefits of insect consumption in both healthy and obese states. In addition, the safety of consuming insects as a unique source of protein, replacing more conventional protein sources (beef-based diet or casein-based diet), was demonstrated.

In the healthy situation, the animals' food intake did not change between groups, and no changes were observed in the tissue measurements or in the percentage of body weight gain. Similarly, a previous study in a healthy rat model in which meat was partially replaced with Alphitobius diaperinus reported no difference in food intake and body weight [3]. Furthermore, our results in the obesogenic state showed that the consumption of insect along with the cafeteria diet did not change kilocalorie intake or the weights of different organs compared to the cafeteria group. These results seem to indicate that insect consumption is similar to other animal protein sources. Interestingly, the insect-based diet in obesogenic state resulted in an increase in small intestine length, in agreement with a previous study in broilers fed Tenebrio molitor larvae [20]. They suggested that this could be a response to chitin content, hypothesising that it could affect the digestibility of protein. Feeding a poorly digestible diet could trigger compensatory mechanisms as the organism attempts to increase the surface area available for nutrient absorption [21]. On the other hand, as expected and previously described [22], morphological assessments highlighted the detrimental effects of cafeteria diet on adiposity and organ weights, underscoring the pathological consequences of prolonged unhealthy dietary habits.

At the metabolic level, in terms of glucose, cholesterol and insulin levels, similar to what has been reported previously [7], we did not observe any changes between the insect group and the control group in the healthy state, reinforcing their healthier metabolic response. The consumption of the beef-based diet increased plasma triglyceride levels compared to the control diet, but they were not altered by the insect-based diet. A previous article also reported an increase in triglycerides and other markers of lipid metabolism when mice consumed beef instead of casein as a protein source [23]. Insects are known to be rich in fatty acids, and although the diets were prepared to achieve the same nutritional profile, the source of protein and fats was changed between the diets. Therefore, these findings highlight the importance of considering the source of protein in dietary interventions aimed at optimising metabolic health.

In the context of obesity, rats exhibited pronounced metabolic dysregulation, characterised by increased caloric intake and dyslipidaemia. The comparison of the healthy and pathological situations revealed distinctive patterns of food intake, with notable variations in macronutrient consumption. While carbohydrate and protein intake remained consistent across the control and cafeteria groups, rats fed a cafeteria diet exhibited reduced fibre intake coupled with increased fat consumption. They consumed more milk and feed, which are a good source of fats, than the other cafeteria food. This results in elevated plasmatic triglyceride levels, as well as a profile of increased NEFAs, glucose, urea, and insulin. Despite the triglycerides being statistically indistinguishable between cafeteria groups, they were selected as the principal variables of separation between these groups. We could observe a compensatory effect of cafeteria-induced changes when animals consumed an insect-based diet. Similarly, chronic supplementation of mice fed a high-fat diet with insect larvae resulted in a reduction in lipid accumulation in the liver and plasmatic levels, as well as a reduction in body weight gain and adipose tissue weight [24]. Furthermore, some authors have previously suggested that the consumption of insects could reduce the intestinal absorption of lipids from the diet due to their bioactive peptides or chitin content [25]. In a separate study, ethanolic extracts from crickets demonstrated the ability to counteract the damage induced by a high-fat diet in rats, resulting in a reduction in abdominal and epididymal fat weight, cholesterol, and triglyceride levels [26]. Collectively, these findings suggest that the consumption of insects may lead to an improvement in the altered metabolic response.

Continuing with the changes at metabolic level, urinary urea was identified as a key variable for discriminating between groups in all comparisons. In light of this, we proceeded to also examine plasma urea levels and the urine-to-plasma ratio. In the healthy state, the beef group exhibited lower urinary urea levels and a lower urine/plasma urea ratio than the control group. Conversely, the plasmatic levels were higher in the beef group than in the insect group. Furthermore, in the pathological state, animals consuming a CAF diet alone also exhibited lower urinary urea levels and a lower urine/plasma urea ratio than the control group. However, the insect-based diet in this situation returned urea to control levels, decreasing levels in plasma and increasing levels in urine. Plasma urea levels vary depending on protein intake, the ability to catabolize protein and adequate renal excretion of urea [27]. Previous research, such as that by Syauqy et al, has shown that the consumption of processed food/sweets and meat-seafood-eggs diets are associated with higher plasma urea levels [28], which are associated with a decline in kidney function [29]. In this sense, beef and cafeteria consumption may affect the renal function, but further determinations are needed. In contrast, considering that protein intake did not change between groups and that urinary urea levels were higher in the insect group, our results may indicate good renal function when insects are consumed and an ameliorative effect of insect consumption on renal function in an obesogenic situation. Another hypothesis is that amino acid metabolism and the urea cycle may be more efficient in an insect-based diet. As previously described by Hermans et al., both mealworm protein and milk-derived protein ingestion are followed by rapid protein digestion and amino acid absorption [7]. However, in general, animal sources are known to have high protein digestibility [30]. Therefore, further studies on ileal amino acid absorption, metabolism and renal function after insect ingestion may be of interest in the near future.

From the integrative analysis perspective, immune and allergenic-related parameters, as well as taste receptor expression, emerged as key indicators distinguishing between a pure protein consumption and insect or beef groups. However, when we observed univariate analysis, only the consumption of insect-based diet elicited significant reduction of TNF-α levels, suggesting a potential immunomodulatory effect. This is in the line of another study which reported that 14-days cricket consumption in healthy adults, reduced systemic inflammation by decreasing plasmatic TNF-α levels [31].

In the diet-induced obesogenic condition, IL-18 and IL-10 were the only that changed between control and cafeteria groups. Similarly, recent study in mice reported changes in IL-18, but not in TNF-α, IL-1β or IL-10 that resulted unaltered when animals consumed western diet [32]. Our study indicated an amelioration of this inflammatory with insect consumption attenuating cafeteria diet-induced IL-18 increase, as well as IL-10, intestinal secretory IgA and intestinal permeability profile which also appear to be returned to homeostatic levels. Considering that inflammation is an important element during the development of obesity or metabolic dysregulations induced by cafeteria diet [33], our results suggest a suppression by insect consumption of pro-inflammatory factors in this context. Insects are known to provide essential unsaturated fatty acids (ω-3 and ω-6), their protein hydrolysis could result in specific peptides, and they are a source of phytosterols that they obtain from dietary sources [34,35]. These nutrients have been widely associated with anti-inflammatory properties [36]. In addition, insects are a source of chitin, and by-products obtained through the action of microbiota could also exert immunomodulatory properties [37]. Thus, there is some indication of an anti-inflammatory response when insects are consumed by animals in both healthy and pathological situations.

Following with the intestinal taste receptor expression, they have important functions in maintain general health [38]. The role of these receptors when located in the apical side of enterocytes is to be activated by the taste components of the diet, particularly umami compounds stimulate tas1r1/tas1r3 heterodimer and bitter compounds stimulate different tas2r [39,40], and trigger metabolic and inflammatory responses. In particular, their activation has been described to be involved in the enteroendocrine secretion of hormones for satiety and metabolic response (ghrelin, GLP-1, CCK, etc) [39]. Furthermore, they have been recently associated to inflammatory regulation [41,42]. Their mRNA expression levels modulation by diet is not extensively studied, but some studies suggested a modulation by agonist or other food components [43].

In the current work, ileal expression of tas2r119 was lower when animals consumed beef or insect diets, compared with casein diet. Moreover, although no significative changes were observed, tas1r1 expression in various locations (jejunum, ileum, and ascending colon) were selected as variable distinguishing healthy groups of rats. We previously observed upregulation of colonic tas1r1 when rats were supplemented by insects (results accepted to be published in Journal of Agricultural and Food Chemistry). Here, despite the absence of significant difference, if we observed the relative expression profile, jejunal and ileal expression of this gene was lower, and colonic expression seemed to be increased in the insect-based diet compared to the beef-based diet. As we previously described, the expression of these receptors is modulated by amino acid composition. Therefore, we could attribute the possible variation of the gene expression to the differentiate amino acid that reach each part of the colon.

Lanng et al. described that ingestion of insect protein was associated with a larger amount of aspartate, methionine, glutamate, branched-chain amino acids (BCAA) and the aromatic amino acids, tyrosine and phenylalanine, in the small intestine, compared with ingestion of pork protein [3]. Dai et al. also described that protein intake resulted in a rise in plasma amino acid concentration; leucine, BCAA and essential amino acids (EAA) concentration was greater for cricket protein, while non-essential amino acids (NEAA) and total amino acids (TAA) concentration was greater for beef-derived protein [8]. Collectively, different pattern of protein residues that reach the intestinal tract and may be absorbed could also affect in a different manner the intestinal response, and particularly, the expression of taste receptors susceptible to certain amino acids.

Moreover, the analysis of intestinal taste receptor expression in pathological condition revealed intriguing insights into the potential of insect-based diet in mitigating cafeteria diet-induced alterations. While cafeteria diet seemed to perturb the expression of umami taste receptors in the jejunum and ascending colon, insect consumption exerted a modulatory effect, particularly evident in the downregulation of jejunal tas1r expression. Although our results not showed significant changes when animals consumed cafeteria which respect to control, previous study with mice revealed an increase of tas1r3 expression after long-term consumption of Western diet and associated this receptor with intestinal inflammatory response [44]. In this context, our study demonstrated that insect-based diets mitigated the potential upregulation of this receptor in the jejunum. Consequently, insect consumption appears to exert a regulatory influence on the expression of taste receptors, which could potentially elicit a range of beneficial physiological responses that must be further investigated.

In conclusion, the present work provides some of the first evidence of the physiological effects of consuming an insect-based diet as a unique protein source compared to traditional protein sources under both healthy and obesogenic conditions. The effects studied in the healthy state, at the metabolic, inflammatory, morphological, and intestinal taste receptor expression levels, underline the potential of insect-based diets as a viable alternative for health promotion. In the context of an obesogenic environment, the consumption of insect-based diets appeared to mitigate some of the adverse effects induced by the cafeteria diet at the levels of inflammation, metabolism, and intestinal taste receptors. This indicates that insects could be a viable alternative protein source, even when used as the main protein contributor in the diet. Furthermore, possibly due to their rich content of bioactive compounds that may confer health advantages [46], their potential benefits extend to pathological conditions such as obesity. Further research is needed to elucidate the underlying mechanisms and long-term effects of insect-based diets.

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## Supplementary Information

**Supplementary Table 1.** Nutritional composition of Control, Beef and Insect diets. SFAs are saturated fatty acids; MUFAs are monounsaturated fatty acids; PUFAs are polyunsaturated fatty acids; and Kcal/g are Kilocalories per gram.

| | Control | Beef | Insect |
|---|---|---|---|
| Protein, % by weight | 14.2 | 14.2 | 14.2 |
| Carbohydrate, %by weight | 63.8 | 64.6 | 62.7 |
| Fat, %by weight | 7.0 | 6.9 | 6.8 |
| SFAs, % of fat | 36.5 | 36.5 | 35.4 |
| MUFAs, % of fat | 32.0 | 32.2 | 32.2 |
| PUFAs, % of fat | 31.6 | 31.4 | 32.3 |
| Kcal/g | 3.7 | 15 | 15.4 |

**Supplementary Table 2.** Other biochemical parameters analysed in Control, Beef and Insect groups. Results are presented as the mean ± SEM (n=7/8).

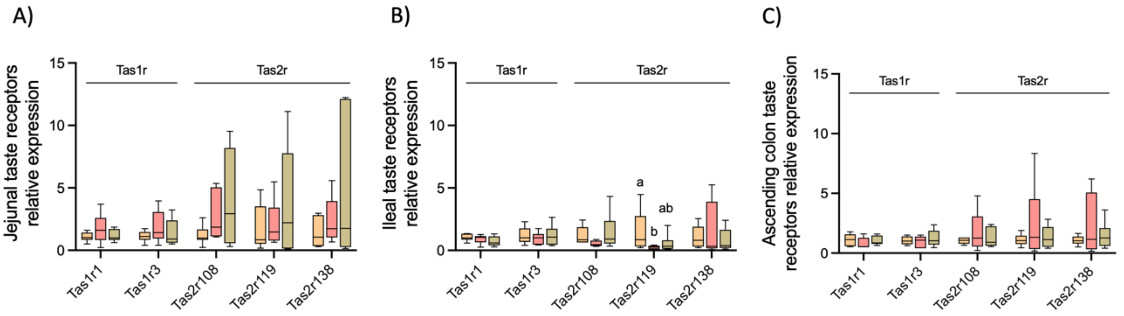
| | Control | Beef | Insect |
|---|---|---|---|
| Glucose (mM) | 7.18 ± 0.33 | 6.83 ± 0.20 | 6.96 ± 0.43 |
| NEFAs (mM) | 0.55 ± 0.04 | 0.59 ± 0.06 | 0.57 ± 0.06 |
| Cholesterol (mM) | 1.99 ± 0.13 | 1.96 ± 0.27 | 2.14 ± 0.10 |
| Insulin (ng/mL) | 2.59 ± 0.25 | 3.00 ± 0.34 | 2.16 ± 0.37 |

**Supplementary Table 3.** Other parameters related to plasmatic and intestinal inflammation, allergenicity, and intestinal permeability, analysed in Control, Beef and Insect groups. Results are presented as the mean ± SEM (n=7/8).

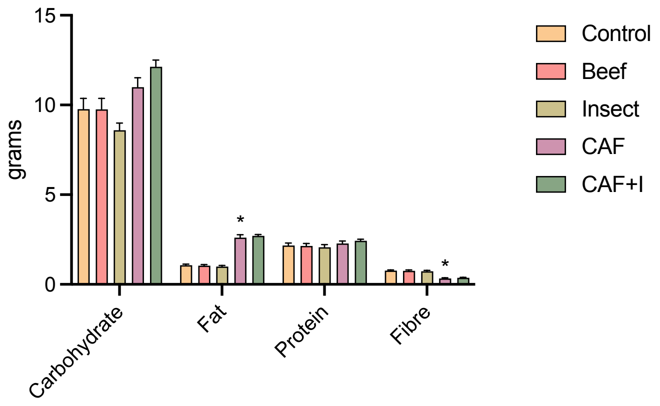
| | Control | Beef | Insect |
|---|---|---|---|
| Plasmatic IL-1β (pg/mL) | 4.70 ± 1.43 | 6.04 ± 1.61 | 5.65 ± 0.95 |
| Plasmatic Histamine (ng/mL) | 11.9 ± 1.44 | 10.9 ± 0.83 | 10.7 ± 0.82 |
| Plasmatic OVA (pg/mL) (intestinal permeability) | 117 ± 15.8 | 104 ± 11.0 | 108 ± 17.4 |
| Intestinal sIgA (ng/mL) | 20.3 ± 0.98 | 18.2 ± 2.03 | 20.7 ± 1.70 |
| Ileal expression of sIgA i.p. | 1.04 ± 0.11 | 1.16 ± 0.14 | 1.41 ± 0.28 |
| Ileal expression of IL-1β | 1.09 ± 0.19 | 1.75 ± 0.37 | 1.09 ± 0.23 |

**Supplementary Table 4.** Morphological parameters analysed in Control, Beef and Insect groups. Results are presented as the mean ± SEM (n=7/8).

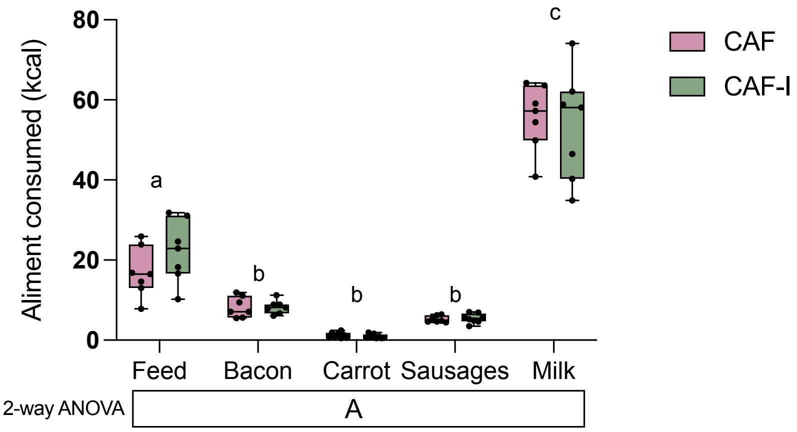
| | Control | Beef | Insect |
|---|---|---|---|
| Body weight gain (%) | 23.4 ± 2.91 | 26.7 ± 2.65 | 21.9 ± 3.31 |
| Adipose tissue (%) | 5.80 ± 0.39 | 5.91 ± 0.47 | 4.87 ± 0.24 |
| Brown adipose tissue (g) | 0.56 ± 0.05 | 0.66 ± 0.07 | 0.55 ± 0.03 |
| Liver weight (g) | 8.01 ± 0.46 | 8.61 ± 0.43 | 8.34 ± 0.51 |
| Kidney weight (g) | 0.80 ± 0.03 | 0.84 ± 0.02 | 0.84 ± 0.03 |
| Stomach weight (g) | 1.20 ± 0.02 | 1.26 ± 0.04 | 1.21 ± 0.04 |
| Spleen weight (g) | 0.70 ± 0.02 | 0.65 ± 0.03 | 0.74 ± 0.05 |
| Full caecum weight (g) | 2.28 ± 0.17 | 2.30 ± 0.07 | 2.37 ± 0.20 |
| Clean caecum weight (g) | 0.73 ± 0.05 | 0.71 ± 0.02 | 0.71 ± 0.03 |
| Small intestine weight (g) | 6.61 ± 0.25 | 7.05 ± 0.32 | 6.42 ± 0.22 |
| Small intestine length (cm) | 101 ± 1.86 | 102 ± 1.18 | 101 ± 2.20 |
| Colon weight (g) | 1.10 ± 0.05 | 1.12 ± 0.05 | 1.10 ± 0.11 |
| Colon length (cm) | 16.1 ± 0.48 | 15.6 ± 0.40 | 16.3 ± 0.95 |



**Supplementary Figure 1.** Taste receptor expression at jejunum (A), ileum (B) and ascending colon (C) of healthy animals. The data are presented in a box and whiskers plot. Boxes represent the median and interquartile range, while whiskers extend from the smallest to the largest values, encapsulating the full range of the data (n=7/8). Different letters indicate differences using Tukey's multiple comparison test ($p < 0.05$).



**Supplementary Figure 2**. Carbohydrate, Fat, Protein, and Fibre intake of each group. Results are presented as the mean ± SEM (n=7/8).



**Supplementary Figure 3.** Kilocalories obtained of each aliment consumed in cafeteria groups. Results are presented as the mean ± SEM (n=7/8). CAF indicates cafeteria; CAF-I indicates cafeteria + insect-based diet group. A indicates significant differences among foods using two-way ANOVA, and letters indicate differences using Bonferroni's multiple comparison test ($p < 0.05$). No differences between CAF and CAF-I groups.

**Supplementary Table 5.** Other biochemical parameters analysed in Control, CAF and CAF-I groups. Results are presented as the mean ± SEM (n=7/8). * indicates statistical difference observed when compared to CAF group using Mann-Whitney ($p < 0.05$).

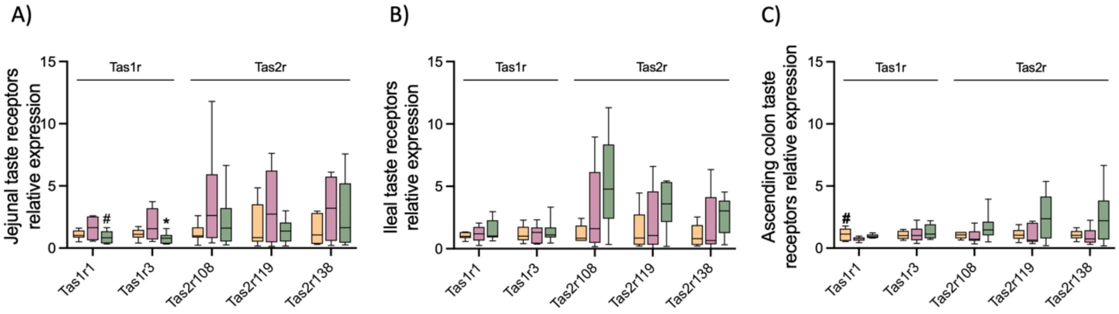
| | Control | CAF | CAF - I |
|---|---|---|---|
| Cholesterol (mM) | 1.95 ± 0.32 | 2.44 ± 0.32 | 2.65 ± 0.19 |
| Glucose (mM) | 7.18 ± 0.33 | 7.78 ± 0.26 | 7.45 ± 0.12 |
| NEFAs (mM) | 0.55 ± 0.04 | 0.67 ± 0.05 | 0.57 ± 0.07 |
| Insulin (ng/ml) | 2.42 ± 0.63 | 3.99 ± 0.56 | 3.80 ± 0.72 |

**Supplementary Table 6**. Other parameters related to plasmatic and intestinal inflammation, allergenicity, and intestinal permeability, analysed in Control, CAF and CAF-I groups. Results are presented as the mean ± SEM (n=7/8).

| | Control | CAF | CAF - I |
|---|---|---|---|
| Plasmatic TNF-a (pg/mL) | 137 ± 30.1 | 191 ± 69.5 | 149 ± 54.3 |
| Plasmatic IL-1β (pg/mL) | 4.70 ± 1.43 | 13.3 ± 4.12 | 15.9 ± 4.88 |
| Plasmatic Histamine (ng/mL) | 11.9 ± 1.44 | 11.0 ± 0.93 | 11.6 ± 1.16 |
| Ileal expression of sIgA i.p. | 1.04 ± 0.11 | 1.77 ± 0.39 | 2.13 ± 0.54 |
| Ileal expression of IL-1β | 1.09 ± 0.19 | 1.51 ± 0.42 | 1.42 ± 0.31 |
| OVA plasmatic levels (pg/mL) (intestinal permeability) | 117 ± 15.8 | 135 ± 15.8 | 131 ± 15.4 |

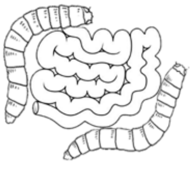
**Supplementary Table 7.** Morphological parameters analysed in Control, CAF and CAF-I groups. Results are presented as the mean ± SEM (n=7/8). # indicates tendency and * indicates statistical difference observed when compared to CAF group using Mann-Whitney (0.1>p<0.05; and p < 0.05 respectively).

| | Control | CAF | CAF - I |
|---|---|---|---|
| Spleen weight (g) | 0.70 ± 0.02 | 0.71 ± 0.02 | 0.74 ± 0.04 |
| Full caecum weight (g) | 2.28 ± 0.17 | 2.07 ± 0.15 | 2.16 ± 0.09 |
| Clean caecum weight (g) | 0.73 ± 0.05 | 0.71 ± 0.06 | 0.77 ± 0.05 |
| Colon weight (g) | 1.10 ± 0.05 | 1.04 ± 0.05 | 1.07 ± 0.06 |
| Colon length (cm) | 16.1 ± 0.48 | 15.9 ± 0.43 | 15.8 ± 0.67 |



**Supplementary Figure 4**. Taste receptor expression at jejunum (A), ileum (B) and ascending colon (C) of CAF animals. The data are presented in a box and whiskers plot. Boxes represent the median and interquartile range, while whiskers extend from the smallest to the largest values, encapsulating the full range of the data (n=7/8). # indicates tendency and * indicates statistical difference observed when compared to CAF group using Mann-Whitney (0.1>p<0.05; and p < 0.05 respectively).

# GENERAL DISCUSSION



# GENERAL DISCUSSION

The exploration of insect consumption as a sustainable protein source has received considerable attention. In this context, the current work aimed to analyse the impact of insect consumption on gut health and subsequently on local and systemic immune and metabolic responses, examining both healthy and induced inflammation scenarios. Furthermore, we explored the potential modulation of intestinal taste receptors by insects and its potential implications for health. To this end, three studies have been carried out to identify the effects of two different insect species: the yellow mealworm (*Tenebrio molitor*) and the lesser mealworm (*Alphitobius diaperinus* or Buffalo). Both belong to the Tenebrionidae family of the order Coleoptera and share similar nutritional compositions at the larval stage [1]. While *T. molitor* is a widely used insect across various sectors and considered promising in the food and feed industries [2], *A. diaperinus* has been less commonly employed, necessitating further studies on their health effects [3]. To fill this gap, we assessed the effects of both *T. molitor* and *A. diaperinus*, to also determine if the effects were species specific. Both insects received a positive opinion from EFSA to be accepted as novel foods for human consumption, however, as mentioned before, further studies on the health effects of their consumption are still needed [4,5]. Additionally, we also compared these effects with those of other conventional protein sources, including high quality animal protein (beef), a fibre-rich vegetable protein (almond) and casein (cow).

First, we analysed in humans the intestinal immune response to insect exposure compared to other more commonly ingested protein sources in human diet. We therefore assessed the *ex vivo* effects of three different protein sources, beef, almond and *A. diaperinus*, on the human intestinal immune response in colon samples. Our results showed that the exposure to insect extracts resulted in lower secretion of inflammatory cytokines and allergenic immunoglobulins compared to almond or beef extracts (**Manuscript 1**). While it has been suggested that high red meat consumption can alter gut microbiota, impair the barrier integrity, and induce the production of inflammatory cytokines [6], the response to almond appeared to be counterbalanced by an anti-inflammatory effect, which has also been described to have a protective effect against the development of gastrointestinal inflammation [7]. With regard to the effect of *A. diaperinus*, our results showed that it did not affect intestinal inflammation, suggesting a healthier inflammatory profile for insect-derived flours compared to other extracts. In this sense, previous studies have highlighted anti-inflammatory properties of insect bioactive compounds [8]. However, to fully assess these effects, further research is needed that examines different concentrations of insect extracts, analyses additional biomarkers and uses a variety of experimental models.

With this in mind, we then carried out an experiment in which Wistar rats were supplemented with chronic low doses of the two insect species. After confirming the absence of adverse health effects in the animals, another study was conducted using chronic insect consumption (*A. diaperinus*) as the only source of protein in the diet, which meant that the insect intake was about 30 times higher than in the supplementation study and compared to the same amount from other protein sources. In addition, both studies included two models: healthy and inflammation-induced status either by intraperitoneal injection of LPS or by cafeteria diet ingestion (CAF diet).

As previously demonstrated, intraperitoneal LPS injection in laboratory rats is described to model an acute intestinal dysfunction, increasing permeability, and causing a pro-inflammatory state in the intestine and systemically [9]. In addition, the cafeteria diet for rats, equivalent to the Western diet in humans, is characterised by being high in refined carbohydrates, simple sugars and saturated fatty acids and low in fibre. This diet in rats mimics the development of the metabolic syndrome in humans, from the onset by promoting hyperphagia to the development of all metabolic symptoms [10,11]. Like LPS model, although through different pathways, cafeteria diet has been linked to the induction of intestinal dysfunction, low-grade inflammation and metabolic endotoxemia [12]. In this sense, in the first study we evaluated the preventive effect of low-dose insect supplementation in an LPS model, and the second study allowed us to analyse the insect consumption as unique protein source in an obesogenic condition. This enabled us to investigate the potential bioactivity of insect consumption in these conditions, providing valuable insights into their effects on intestinal health and inflammation.

Starting with the intestine, due to its importance as the primary barrier between external food and pathogens and the body, the morphology from duodenum to colon was mainly preserved with insect supplementation. Notably, a higher villus/crypt ratio was observed in the jejunum. In contrast, LPS injection, as previously described by other authors [13,14], decreased the ileal villus/crypt ratio. However, this effect was mitigated by insect supplementation (**Manuscript 1**). The structure of crypts and villi is a very important element of the intestinal epithelium, indicating the proliferation and absorption of intestinal cells and contributing to its homeostasis [15]. While an increase in the villus/crypt ratio is associated with improved intestinal digestive and absorptive functions and is an indicator of intestinal health, maturity and enterocyte capacity, poor intestinal development can be associated with various diseases and reduced nutrient absorption [16,17]. In line with this, we calculated the absorptive surface area. Although in the univariate analysis there were no statistical differences in this measure as a result of insect consumption, the results of the integrative analysis presented in **Manuscript 2** placed this determination as a key variable distinguishing between the insect-supplemented and non-insect-supplemented groups. Specifically, the absorptive surface area of the three segments of the small intestine were selected, positioning this variable as the most affected by the insect-supplemented groups, similar to what was recently described by Vasilopoulos *et al*. Similarly, as shown in **Manuscript 3,** an increased gut length was observed when animals consumed insect-based diets in an inflammatory state, also indicating an expansion of the absorptive surface area. Recent studies have shown similar effects to ours when pigs or chickens consume insect larvae [15,16]. Certain components present in insects, such as bioactive peptides and specific amino acids, can stimulate the secretion of intestinal growth factors and contribute to improved intestinal morphology [17]. Furthermore, this effect could be explained by the content of chitin and its derivatives in insects, which has been postulated to also increase the absorptive surface [13,18,19]. Taken together, these studies suggest that the insect consumption contributes to intestinal development, health, and functionality.

Following with the effects of insect intake in the intestinal system, neither the low-dose of insect supplementation nor the insect-based diet did not disrupt immune barrier on the mucosal immune response in healthy or inflammatory-induced conditions. This was first observed by measuring secretory IgA (sIgA) in intestinal lavage fluids. sIgA plays a crucial role as the first line of defence on mucosal surfaces against infectious microorganisms and toxins. Therefore, its presence and activity are critical in maintaining the integrity of the mucosal barrier and preventing the entry of harmful agents into the bloodstream [20]. This immunoglobulin was not altered when rats consumed insect flour as a supplement or as a sole protein source in the healthy state. On the contrary, although not statistically significant, intestinal sIgA levels or gene expression, which appeared to be increased in both inflammatory models compared to the healthy state, were also selected by integrative analysis to differ between insect and non-insect feeding groups (**Manuscript 2**). Here, the rats that consumed insects appeared to have lower levels than the inflamed rats (**Manuscript 1** and **3**). In addition, we assessed changes in intestinal permeability by measuring plasmatic levels of ovalbumin (OVA), which is derived from the OVA test performed [21]. It only increased in LPS-treated animals, confirming the expected effect of LPS, thereby indicating that insect consumption did not affect intestinal permeability of rats (**Manuscript 1**). In contrast, although CAF-diet is a good model for intestinal disruption and increased permeability, here we did not find significative changes after 12-weeks of CAF diet. We suspected that a longer duration of this diet might result in greater increase of permeability, as suggested by our previous studies, where this effect was more pronounced after 17 weeks [21]. However, despite the previous limitations, it can be concluded that insect consumption did not significantly impact intestinal permeability in this context. Overall, these findings suggest that insect consumption does not compromise intestinal integrity or immune function, supporting its potential as a sustainable protein source with beneficial effects on gut health.

All of the above findings are consistent with the broader understanding of how dietary choices affect intestinal function. In terms of nutrient sensing, the gastrointestinal tract can sense umami, sweet and bitter tastes in much the same way as the tongue through GCPRs taste receptors [22–24]. Known for their role in sensory perception, taste receptors also play an important role in regulating gut function [25]. As previously observed, umami and several bitter taste receptors could be modulated by dietary components [26–28]. Therefore, knowing whether insect consumption modulate these taste receptors is crucial to understanding their potential impact on gut health and overall well-being. In this sense, the current thesis also analysed the effects of insect ingestion on the expression of intestinal taste receptors and their implication in intestinal and general health. Regarding the relative gene expression of these receptors, we identified colonic Tas1r1, together with Tas1r3 and some bitter taste receptors spanning from the jejunum to the colon, as receptors susceptible to modulation by low dose insect supplementation (**Manuscript 2**). In particular, the expression of Tas1r1 and Tas1r3, that form the heterodimer of the receptor for umami tastes primarily L-amino acids and peptides, was significantly increased in the ascending colon when animals were supplemented with insects. In this sense, numerous studies have demonstrated that chronic exposure to certain dietary compounds, including taste receptor agonists, can lead to changes in the mRNA expression of these receptors [29–31]. Moreover, dietary consumption of glutamate and the branched-chain amino acids (BCAAs), isoleucine, leucine, and valine, has been shown to upregulate jejunal umami receptors expression [32,33]. Knowing that mealworms are a good source of glutamate and aspartate, an also contain BCAAs, the modulation might be through the differentiating amino acids that reach the colon when animals are supplemented with insects. Nevertheless, when the chronic consumption of insects was about 30 times higher than in the first study and the unique source of protein (**Manuscript 3**), the umami ones were not significantly changed as previously observed. However, looking at the relative expression profile, jejunal and ileal expression of the Tas1r1 gene was lower, while colonic expression was increased in the insect-based diet compared to the beef-based diet. Previous studies showed that different types of amino acids are absorbed when compared insect-protein ingestion with others animal-derived protein [34,35]. In this sense, it is again suggested that some variations in the expression of taste receptors could be explained by different patterns of protein residues reaching and being absorbed in each section of the intestine. In addition, the intestinal microbiota and the SCFA products resulting from their activity have also been shown to modulate taste receptor expression [36], suggesting a multifaceted mechanism involving not only amino acid composition but also microbial-derived metabolites in colonic taste receptor modulation.

On the other hand, as some of these bitter receptors, including Tas2r108, Tas2r119, Tas2r138, Tas2r139, and Tas2r143, have also been shown to be sensitive to certain peptides and amino acids [37–41], we suspected that their expression could be also modulated by insect consumption. In this sense, the buffalo-supplemented group showed significantly lower expression levels of colonic Tas2r119 and Tas2r138 (**Manuscript 2**), reinforcing the idea that there is a specific modulation of taste receptors in the colon that may be influenced by the microbiota [42,43]. Conversely, jejunal Tas2r119 also emerged as a key variable that facilitated the differentiation between the insect-supplemented groups and the control group. Although these receptors lost their importance in distinguishing the groups when the animals consumed insects as their only source of protein, some of them, such as ileal Tas2r119, changed their expression levels between the groups of healthy animals (**Manuscript 3**). This seems to indicate that Tas2r119 is the bitter taste receptor that is most susceptible to modulation by insect consumption.

Taste receptors, among different physiological functions have been implicated to inflammatory response [44,45]. Additionally, in pathologies associated to a low-grade inflammation, such as obesity, evidence suggests that taste perception is altered and that taste receptors are involved in regulating inflammatory status [46]. Thus, in the context of inflammation, taste receptors may be affected and potentially contribute to the immune response, thus creating a feedback loop [47]. In this sense, in **Manuscript 1** we observed that the intestinal expression of taste receptors, again especially Tas1r1, Tas1r3 and Tas2r119, have been altered by the LPS injection, which help to establish a connection between taste receptors and immune disturbances, as has previously been described for both Tas1r and Tas2r [48,49]. Nevertheless, in this condition, insect consumption did not affect taste receptors expression, possibly because a too low dose of insect. On the contrary, in the obesogenic condition, we only observed a tendency of downregulate the colonic Tas1r1 expression in CAF rats, and insect-based diet consumption seemed to counteract this reduction (**Manuscript 3**). As in the healthy situation, jejunal umami taste receptors were downregulated with insect consumption in this obesogenic situation, and always seemed to modulate their expression in the same direction as healthy controls, counteracting the cafeteria effects.

All this evidence supports the idea that the expression of gut taste receptors is highly sensitive to changes in dietary patterns and health status. In particular, we demonstrated that insect consumption modulates the expression of several intestinal taste receptors. The next step was therefore to know the possible consequences of this modulation on physiological functions, as taste receptors have been linked to important ones [23]. For example, the activation of bitter taste ones has been described to be involved in the enteroendocrine secretion of hormones for satiety and metabolic response (ghrelin, GLP-1, CCK, etc) [50]. Interestingly, some authors have also shown that the intestinal secretion of the satiety hormone CCK, can occurs through the Tas1r1/Tas1r3 activation, while others have also suggested GLP-1 secretion after amino acid activation of this receptor [33,51]. In line with this, we previously observed an increase in plasma GLP-1 levels after the insect supplementation [1], and the integrative analysis performed in the **Manuscript 2** revealed that GLP-1 is another key variable in distinguishing the insect-supplemented and control groups of animals. Taken together, our results provide more evidence of a possible relationship between umami receptors (Tas1r1/Tas1r3), both modulated by insect ingestion, and hormone secretion in the intestine. Furthermore, extra-oral taste receptors have been recently associated to inflammatory regulation, colonic motility, and metabolic function [46,48,52], but more studies on the possible role in the intestine and beyond it are needed. In this sense, our correlations study revealed that colonic Tas1r1, upregulated by insect ingestion, showed a negative correlation with goblet cells. Although previous studies have shown that goblet cells express bitter taste receptors in their membrane [53], for the moment, Tas1r types have only been described in enteroendocrine and colonic tuft cells [54,55]. In this sense, one possible explanation is that the activation of Tas1r1 receptors may trigger downstream signalling cascades that modulate goblet cell development. Additionally, positive correlations were found between ileal bitter and umami taste receptors and ileal secretory IgA expressions, emphasizing the role of taste receptors in the intestinal immune response [48]. Finally, jejunal Tas2r119, the bitter most modulated by insect consumption and LPS, indicated negative correlation with intestinal permeability, similarly as previously described in an *in vitro* study [56]. These findings collectively set the basis for future research to explore the specific involvement of taste receptors in intestinal function, specifically barrier integrity and immune response, both in healthy and pathological conditions.

On the other hand, in view of all the above, and given the close relationship between intestinal health and its potential influence on overall well-being, possibly through modulation of taste receptors, we also focused on investigating systemic parameters to study the impact of insect consumption on overall health. In this sense, we also carried out a screening of systemic parameters. Firstly, as the current concern about insect consumption is the potential allergic reactions, we wanted to assess the allergenic response. Under healthy conditions, we did not observe any allergenic response after insect consumption. More interestingly, however, was the study in the context of intestinal dysfunction, such as in LPS-treated or obese animals, because several studies have reported that increased permeability of the intestinal epithelium may facilitate the entry of potential food allergens, thus increasing sensitisation and allergy risk [57,58] . Our results provide the first description of the evidence of a non-allergenic effect of low-dose insect supplementation (**Manuscript 1**), and of insect-based diet (**Manuscript 3**), in impaired permeability and intestinal inflammation animal models (**Manuscript 1**). The allergic response typically requires an initial pre-sensitisation followed by a subsequent interaction with the allergen to trigger the allergic response [59]. In this sense, our chronic and continuous insect supplementation in both studies takes this into account.

Continuing at the immune level, while our first study showed no significant changes in systemic inflammatory cytokine levels when animals were supplemented with a low dose of insects, either in healthy or in LPS-treated rats (**Manuscript 1**), our second approach showed that consumption of the insect-based diet affected the systemic immune response (**Manuscript 3**). Under healthy conditions, consumption of the insect-based diet induced a significant reduction in plasma TNF-α levels, suggesting a potential systemic anti-inflammatory effect in rats. This was consistent with findings in healthy humans, where 14 days of cricket consumption was reported to also reduce systemic inflammation by decreasing plasma TNF-α levels [60]. Similarly, in the obesogenic situation where a low-grade inflammatory state is induced [10], insect consumption attenuated the cafeteria diet-induced increase in IL-18 and IL-10, which returned to homeostatic levels, suggesting potential anti-inflammatory properties associated with insect-based diets. The insects' content of essential unsaturated fatty acids (ω-3 and ω-6), phytosterols and polyphenols, which they can obtain from their diet, together with the specific peptides derived from their protein, could explain these immune-protective effects [61,62]. In addition, the action of microbiota due to their chitin content could also exert immunomodulatory properties [63].

Thus, our studies indicated an anti-inflammatory response when insects are consumed chronically and in high doses as part of the diet in both healthy and pathological situations.

As mentioned before, the impact of insect consumption on the immune response could also be mediated by intestinal taste receptors. In this sense, some publications have suggested alterations of the taste receptors in immune disturbance situations [64], as well as their participation in maintaining immune balance [65]. In this sense, our correlation analysis in LPS situation showed negative connexion between plasmatic TNF-α, IL-1b and IL-10 levels and bitter taste receptors (especially Tas2r119) in the jejunum as well positive correlation with umami taste receptors (especially Tas1r1 subunit) in the proximal colon (**Manuscript 2**). LPS injection results showed a downregulation of jejunal bitter taste receptors, accompanied by a pronounced upregulation of colonic Tas1r1, which appeared to be attenuated by insect consumption in this pathological condition. This may suggest that the effect of insects on immune regulation parallels the modulation of bitter and umami taste receptors in the intestine. However, the mechanics and impact of these receptors on the body's systemic functions need to be further analysed.

Finally, in terms of metabolic responses, our results showed that insect ingestion had no significant effect on metabolic parameters under healthy conditions, similar to what has been reported previously [66], either with insect supplementation (**Manuscript 1**), or when all the dietary protein from the diet was from insect (**Manuscript 3**). However, in this second dietary intervention, nitrogen metabolism was different from that in the beef group. Regarding the pathological situation, LPS treatment causes metabolic dysregulation, whereas *T. molitor* consumption significantly prevented LPS-induced changes in glycemia and uraemia (**Manuscript 1**). Similarly, rats in the obesogenic state induced by the cafeteria diet exhibited a pronounced metabolic dysregulation characterised primarily by increased calorie intake, dyslipidaemia and uraemia disorders (**Manuscript 3**). Again, insect consumption seemed to alleviate the dyslipidaemia and uraemia disorders in this obesogenic condition. Plasma urea levels vary depending on protein intake, the ability to catabolize protein and adequate renal excretion of urea [67]. In this sense, rapid protein digestion and amino acid absorption following insect ingestion, as described in previous studies [66], may contribute to more efficient urea cycle metabolism. Furthermore, our results on the triglyceride levels are consistent with previous studies suggesting that insect consumption may reduce the intestinal absorption of dietary lipids due to their bioactive peptides or chitin content [68]. Taken together, this evidence confirms that there are no effects of insect consumption that compromise the health of the rats and also suggests that insect-based diets may be metabolically beneficial, as has been recently suggested by Kang *et al* [69]. Interestingly, the same authors found a parallel improve microbiota profile, increasing the diversity and the beneficial bacteria taxa when diet include *T. molitor* and *A. diaperinus* [70], but suggested more investigation in how they may be linked with the metabolic benefits. Another hypothesis could be that the amelioration of inflammatory status, also affect the metabolic status, as TNF-α and interleukins-1 are related to induce metabolic disturbances

[71]. Although our results of **Manuscript 1** do not allow to explain these effects maybe because the low dose of insect, the improvement of IL-10 and IL18 levels in the **Manuscript 3** study supports this hypothesis.

Moreover, in this context, our correlation study also revealed some interesting associations between intestinal taste receptors and biochemically analysed parameters (**Manuscript 2**). Our results support the idea of a possible relationship between intestinal bitter taste receptors and lipid metabolism [72], as triglycerides and cholesterol levels are highly correlated with these receptors. In addition, recent research has furthered our understanding by demonstrating that cholesterol acts as an agonist for bitter taste receptors, affecting their function [73,74]. Together, these findings highlight the intricate interplay between lipid metabolism and taste receptor signalling pathways, potentially opening up new avenues for understanding and treating metabolic diseases. In addition, our study identified correlations between plasmatic levels of creatinine and urea with jejunal umami taste receptors, suggesting possible links between umami taste perception and amino acid metabolism. Dietary glutamate and aspartate are known to be metabolised in the intestine to various products, including urea metabolites [75,76]. Furthermore, our results describing that insect consumption can counteract metabolic changes induced by inflammatory conditions (**Manuscripts 1** and **3**) strengthen the link between L-amino acid levels reaching the jejunum and plasma urea levels. However, the observed correlations also suggest a possible role for umami taste receptors in the regulation of nitrogen metabolism., Taken together, the findings raise intriguing questions about the systemic effects of taste receptor modulation beyond the digestive system. Nevertheless, these assumptions are largely based on our observations and hypotheses derived from the existing literature, and we cannot definitively demonstrate the effects of modulating taste receptor gene expression. Future research will be crucial to demonstrate these relationships and validate the hypotheses presented.

In summary, the results presented in this thesis partially describe the health effects of consuming an unusual protein source, of high quality and environmentally friendly production, with a high feed conversion ratio, high fertility rates and short life cycles [77].

Throughout our study, we uncovered associations between taste receptors and other parameters analysed, laying the groundwork for future investigations into the mechanisms and significance of these relationships. Specifically, we found that insect-based diets may influence the expression of umami and bitter taste receptors in the gut, potentially affecting nutrient sensing, enteroendocrine hormone secretion and inflammatory regulation in the gastrointestinal tract. However, it is important to acknowledge some limitations. Firstly, our analysis was limited to the gene expression level, providing a snapshot of taste receptor activity without assessing protein expression or localisation. Nevertheless, Lipchock *et al.* have reported in two studies that the perception of bitterness is correlated with the abundance of bitter receptor mRNA [78,79], which could indicate that the levels of mRNA of these receptors are highly related to the levels of protein capable of recognising the specific compounds. Moreover, further investigation of the protein expression of taste receptors, particularly at the apical side of enterocytes and at different locations within the intestine, could elucidate the functional implications of the gene expression changes we have observed. Techniques such as Western blotting and immunohistochemistry could complement our mRNA expression analysis and provide insights into protein localisation, abundance and signalling pathways. In addition, our study analysed whole intestinal tissue without specific resolution at the cell level. While this approach provides a broad overview of changes in taste receptor expression, future research could benefit from cell-specific analyses to uncover the precise roles of taste receptors in different cell types within the intestinal tract. For example, studying the expression of taste receptors in enteroendocrine cells, tuft cells or immune cells could provide insights into their functional significance beyond nutrient sensing. In addition, studies such as the recent ones by Shon *et al.* or Feng *et al.* [65,80] which analyse the impact of taste receptors and study their signalling pathways in different situations, could lead to a better understanding of the importance of taste receptors and a good development of new novel therapeutic strategies and dietary interventions in situations of pathology.

On the other hand, our results showed healthy responses in terms of systemic and intestinal inflammation, allergenic response and intestinal morphology in rats after chronic low-dose insect supplementation or insect-based diet consumption. In addition, our results suggest that consumption of an insect-based diet as a protein source could improve some metabolic, inflammatory and intestinal variables altered by low-grade inflammation, as occurs in obesity. Hence, our study provides a comprehensive analysis to demonstrate the health effects of insect consumption in healthy and inflammatory states. However, further studies on the effects of bioactive compounds contained in edible insects on human health are needed to validate the potential of edible insects as a novel measure to combat obesity and promote overall health [81].

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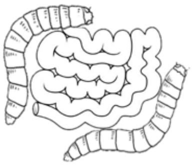
# CONCLUSIONS



# CONCLUSIONS

The main conclusions of the present doctoral thesis are the following:

**1. Chronic low doses of insects cause a healthy response in the intestinal function and overall well-being**

- *A. diaperinus* exerted a healthier intestinal immune response *ex vivo* in human colon than other more common protein sources.
- In healthy rats, insect supplementation did not alter intestinal barrier or systemic metabolic, inflammatory or allergenic parameters.
- Insect supplementation increased intestinal absorption capacity in healthy and LPS-treated rats.
- Insect supplementation did not exacerbate inflammatory, metabolic, or intestinal disruption induced by LPS injection.
- Insect supplementation ameliorated the altered glycemia and uraemia caused by LPS injection.
- The observed effects are not species-specific, since no differences between *A.diaperinus* and *T. molitor* were observed.

**2. Insect supplementation modulates intestinal taste receptors expression, and it is linked to intestinal, metabolic and inflammatory responses**

- Colonic umami and bitter taste receptors are the most sensitive to insect supplementation.
- Colonic Tas1r1 was selected as the key variable to discriminate between control and insect-supplemented groups.
- The expression of taste receptors in different segments of the intestine correlates with several physiological parameters, including local immune response, and systemic metabolic and inflammatory markers

3. A diet based on insects as a unique source of protein leads to a different metabolic and inflammatory profile compared to other protein sources

- In a healthy state, insect-based diets result in different nitrogen and lipid metabolism than beef-based diets.
- Insect-based diets again modulate some jejunal and colonic taste receptors expression.
- Consumption of insect-based diets showed a lower pro-inflammatory profile than diets based on other conventional protein sources, in healthy and obese conditions.
- The insect-based diet alleviated the dyslipidaemia and uraemia disorders induced by the obesogenic condition.

# CONCLUSIONS

Les principals conclusions de la present tesi doctoral són les següents:

## 1. Baixes dosis cròniques d'insectes provoquen una resposta saludable en la funció intestinal i el benestar general

- *A. diaperinus* va exercir una resposta immunitària intestinal *ex vivo* més saludable que altres fonts de proteïnes més comunes en el còlon humà.
- En rates sanes, la suplementació amb insectes no va alterar la barrera intestinal ni els paràmetres metabòlics, inflamatoris o al·lèrgens sistèmics.
- La suplementació d'insectes va augmentar la capacitat d'absorció intestinal en rates sanes i tractades amb LPS.
- La suplementació d'insectes no va exacerbar la disrupció inflamatòria, metabòlica o intestinal induïda per la injecció de LPS.
- La suplementació amb insectes va alleugerir l'alteració de la glucèmia i la urèmia causades per la injecció de LPS.
- Els efectes observats no són específics de cada espècie, ja que no es van observar diferències entre la suplementació amb *A.diaperinus* i *T. molitor*.

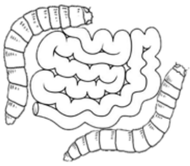
## 2. La suplementació amb insectes modula l'expressió dels receptors intestinals del gust, i està relacionada amb respostes intestinals, metabòliques i inflamatòries

- Els receptors del gust umami i amarg al còlon són els més sensibles a la suplementació amb insectes.
- El Tas1r1 a còlon va ser seleccionat com la variable clau per discriminar entre grups control i suplementats amb insectes.
- L'expressió dels receptors del gust en diferents segments de l'intestí es correlaciona amb diversos paràmetres fisiològics, inclosa la resposta immunitària local, i marcadors metabòlics i inflamatoris sistèmics.

**3. Una dieta basada en insectes com a font única de proteïnes condueix a un perfil metabòlic i inflamatori diferent en comparació amb altres fonts proteiques**

- En un estat saludable, les dietes basades en insectes resulten en un metabolisme nitrogenat i lipídic diferent que les dietes basades en carn de boví.
- Les dietes basades en insectes tornen a modular algunes expressions dels receptors gustatius de jejú i còlon.
- El consum de dietes basades en insectes va mostrar un perfil proinflamatori més baix que les dietes basades en altres fonts proteiques convencionals, en condicions de salut i obesitat.
- La dieta basada en insectes va alleujar els trastorns de dislipèmia i urèmia induïts per la condició obesogènica.

# ANNEX



# ANNEX

## PUBLISHED PAPERS

**Segú, H**.; Jalševac, F.; Pinent, M.; Ardévol, A.; Terra, X.; Blay, M. T. Intestinal Morphometric Changes Induced by a Western-Style Diet in Wistar Rats and GSPE Counter-Regulatory Effect. *Nutrients* 2022, *14* (13), 2608. https://doi.org/10.3390/NU14132608. PMID: 35807788; PMCID: PMC9268310.

Miguéns-Gómez, A.; Sierra-Cruz, M.; **Segú, H**.; Beltrán-Debón, R.; Rodríguez-Gallego, E.; Terra, X.; Blay, M. T.; Pérez-Vendrell, A. M.; Pinent, M.; Ardévol, A. Administration of Alphitobius Diaperinus or Tenebrio Molitor before Meals Transiently Increases Food Intake through Enterohormone Regulation in Female Rats. *J Sci Food Agric* 2023, *103* (4), 1660–1667. https://doi.org/10.1002/JSFA.12305. PMID: 36324158; PMCID: PMC10099498.

**Segú, H**.; Jalševac, F.; Sierra-Cruz, M.; Feliu, F.; Movassat, J.; Rodríguez-Gallego, E.; Terra, X.; Pinent, M.; Ardévol, A.; Blay, M. T. Assessing the Impact of Insect Protein Sources on Intestinal Health and Disease: Insights from Human Ex Vivo and Rat in Vivo Models. *Food and Function* 2024, *15* (8), 4552–4563. https://doi.org/10.1039/D4FO00381K. PMID: 38584501.

## SUBMITTED PAPERS

**Segú, H.**; Jalševac, F.; Lores, M.; Beltrán-Debón, R; Terra, X.; Pinent, M.; Ardévol, A.; Rodríguez-Gallego, E.*; Blay, M.T. Intestinal Taste Receptor Expression and Its Implications for Health: An Integrative Analysis in Female Rats after Chronic Insect Supplementation. Journal of Agricultural and Food Chemistry, 20 January 2024.

Jalševac, F.; Descamps-Solà, M.; Grau-Bové, C.; **Segú, H**.; Auguet, T.; Avilés-Jurado, F.X; Balaguer, F., Jorba, R; Beltrán-Debón, R; Blay, M.T; Terra, X.; Pinent, M.; Ardévol, A. Profiling Bitter Taste Receptors (TAS2R) along the Gastrointestinal Tract and their Influence on Enterohormone Secretion. Gender- and Age-Related Effects in the Colon. Submitted to Plos Biology, 26 April 2024.

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Jalševac, F.; Segú, H.; Balaguer, F.; Ocaña, T.; Moreira, R.; Gràcia-Sancho, J.; Fernandez, A.; Andres-Lacueva, C.; Martínez, M.; Beltrán-Debón, R.; Rodríguez-Gallego, E.; Terra, X.; Ardévol, A.; Pinent, M. TASR5 and TAS2R38 are bitter taste receptors whose colonic expressions play important roles in age-associated processes. Submitted to Molecular Metabolism. 17 May 2024.

## PAPERS UNDER PREPARATION

**Segú, H**.; Lores, M.; Soler, O.; Colom-Pellicer, M.; Jalševac, F.; Beltrán-Debón, R; Terra, X.; Pinent, M.; Ardévol, A.; Rodríguez-Gallego, E.*; Blay, M.T. Examining the Impact of Insect-Based Diets on Intestinal and Overall Health, in Healthy and Diet-Induced Obese Rats.

## LIST OF POSTER AND ORAL COMMUNICATIONS

September 2021 - Participation in the eNuGO Week 2021: Immuno-nutrigenomics: How to feed the immune system – Presentation: Chronic insect administration effect on behavioural, morphometrical, biochemical and immunological parameters in Wistar female rats.

November 2021 – Participation in the I Congreso Internacional en Ciencias Biomédicas – Poster presentation: Efecto de la administración crónica de insecto en los parámetros de comportamiento, morfométricos, bioquímicos e inmunológicos de ratas hembra Wistar.

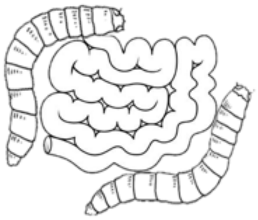
September 2022 – Participation in the NuGO week 2022: Food bioactives for disease prevention – From mechanisms to chrononutrition, Tarragona – Poster presentation: Intestinal Morphometric Changes Induced by a Western-Style Diet in Wistar Rats and GSPE counter-regulatory effect.

April 2023 – Participation in Sessió Científica: On es dirigeix la recerca en nutrició? Tendències i necessitats de la societat, organized by CCNIEC, Barcelona. – Efecte de noves fonts de proteïna (*Alphitobius diaperinus i Tenebrio molitor*) sobre la resposta immunològica intestinal en rates Wistar i humans.

September 2023 – Participation in the Food Bioactives and Health, Prague – Modulation of the expression of umami, sweet and bitter taste receptors in Wistar rats by the insect protein consumption.

November 2023 – Participation in Retreat 2023, organized by IISPV, Arnes, Tarragona – Oral communication: Effect of novel protein sources (Alphitobius diaperinus and Tenebrio molitor) on intestinal immune response in rats and humans.

# ACKNOWLEDGEMENTS



## ACKNOWLEDGEMENTS

En primer lloc, m'agradaria agrair a les meves directores, Mayte i Esther, per l'oportunitat que em vau donar fa 3 anys i pico de formar part del grup MoBioFood, per ajudar-me a créixer com a investigadora i animar-me en tot moment. He après moltes coses de vosaltres, m'heu guiat i donat el suport i la confiança que ha permès aconseguir aquest resultat. Moltes gràcies per tot.

I would like to thank Jamileh for accepting me to do my international stay in Université Paris Cité. You gave me the opportunity to learn new laboratory techniques and the freedom to carry out the experiments for my thesis. I am very thankful to you for facilitating my stay in Paris. Merci beaucoup!!

També agraeixo a la resta de MoBioFood sèniors, Anna, Montse, Raúl i Ximena, el recolzament durant tot aquest temps i tots els bons moments fora de la feina. I ara, a tots els companys i amics que m'emporto, que heu fet que aquests anys estiguin plens d'experiències i aprenentatges. Gràcies Carme, Marta i Alba per haver estat referents per mi. Sou unes cracks i, tot i que el temps que vam compartir no va ser pas tant, vaig aprendre i vaig poder gaudir molt de vosaltres, tan dins com fora del laboratori. M'enduc molts records de sopars, festes i viatges per sempre.

Ara us toca a vosaltres, Maria i Júlia, per ser les millors amigues que m'emporto d'aquesta etapa. Gràcies Júlia per ajudar-me en els meus primers passos al lab, fent la MPO de l'Adrià i aprenent a fer ELISA. Treballar amb tu era molt fàcil i vam congeniar molt ràpid. Va aparèixer la Maria, amb les seves històries sobre Camprodon (l'únic semàfor, el túnel, l'SPAR, la Teisa i la ratafia al Casal), i vam forjar una forta amistat que segur que perdurarà. Gràcies Maria per tots els moments, per acompanyar-me en la bogeria de treballar 12h al dia, carregant 10 plaques de PCRs, venint dies festius per les meves cèl·lules, i ajudant-me tantes i tantes vegades. Són molts els records de les mil hores que hem passat juntes també fora de la uni, al cotxe parlant, mirant futbol, anant a buscar l'Arnau, o anant-nos a vacunar. T'ho agraeixo molt. Moltes gràcies a les dues per ser al meu costat durant aquests anys, per venir a París, pels sopars, vermuts, concerts i viatges, i per tot el que vindrà! Us estimo molt!

Al Florijan, moltes gràcies per també formar part de molts bons moments, per ensenyar-nos Eslovènia, i per ser la meva "parella científica", que així ho has demostrat sempre havent estat al meu costat i sent un bon amic. Gràcies a l'Esther, ara com a amiga, per ajudar-me tant, per tenir converses profundes i cantar *triski-triski-triski* mentre preparem l'article per enviar i pels moments fora de la uni que et fan única. Al Zac, Kuskus!!, moltes gràcies for being a good

partner and friend as well as the best organiser of trips to Kenya. A l'Adrià, per ser el meu nòvio i cuidar-me sempre. Perquè, tot i que, com diu l'Esther, ets un ministre, les teves explicacions i dubtes científics (que creen debats interessats fins i tot en les reunions de grup) et fan ser únic. Estàs inclòs en molts dels millors records que tinc d'aquests anys; per tot això, moltes gràcies! Mònica i Cèlia també formeu part de molts d'aquests bons moments i sense vosaltres no hauria estat el mateix. Gràcies Mònica per sempre estar disposada a ajudar, ser tan treballadora, en moments d'estrès màxim haver-me recolzat i, sobretot, per sempre tenir temes de conversa. Cèlia, tot i que no haguem treballat gaire juntes, sé que ets una crack i que estàs sempre disposada a donar un cop de mà, i fora de la uni ha estat un plaer compartir temps amb tu. A la més nova, Oria, et desitjo el millor i n'estic segura que tot anirà perfecte, perquè has demostrat que ets capaç d'acabar ELISAs desmuntats, a les vuit del vespre i plorant ;), gràcies també per aquestes bones estones! Gràcies Marina, per ser la post-doc referent d'aquests últims mesos, pels teus consells i paciència amb mi. A tots els estudiants que heu passat pel grup, i especialment als últims amb qui més temps d'estrès he compartit, Margalida, Aritz, Luis i Adrià M; la vostra feina i ganes d'aprendre ajuda molt, i personalment us agraeixo els moments compartits. També agraeixo haver conegut gent tan maca com la Francesca o la Marie, sou molt mones. I, tot i que no siguis del grup, com si ho fossis... gràcies Ari per aquest anyet, per aportar calma en un despatx de bogeria, per també unir-te als *salseos* i per el teu suport.

Els Nutris també heu estat molt importants en tot aquest viatge i no em vull deixar a ningú. A tots, gràcies per uns primers estius de platja i per les nits de festa. Al Pauli, el més mono del món, gràcies per ensenyar-me Gérgal, per sempre tenir a punt una abraçada i un somriure que es contagia, per ser tan dolç i bonic. Gràcies Rafiki per sempre voler organitzar excursions i partits de futbol i per, tot i la teva capa de voler ser dur, ser un tros de pa i bon amic. Gràcies Néstor per ajudar-me en tantes coses, pel haver-me descobert el mètode de determinació de nitrits que tu i jo sabem realment com es diu, per guiar-me en el final d'aquesta tesi i per ser com ets! No canviïs mai els teus acudits tontos, que ens animen cada dia. Al Marc, gràcies per descobrir-nos els millors llocs de Mallorca, per les excursions i vies ferrates; ara seré més lliure i en podrem fer més! Al Jorge, per la seva passió pel Mallorca, i per tots els partits de futbol... hi tornem? A d'altres que han format part del meu dia a dia durant aquest temps, algunes que ja han marxat, Ivan, Fra i Vero, i d'altres que han començat recentment, Alba i Aina, ha estat un plaer coincidir amb totes vosaltres.

Niurka i Rosa, moltes gràcies per sempre estar disposades a ajudar i ser un pilar molt important perquè les coses surtin bé, he après molt de vosaltres.

Al Jorge, per haver-me ajudat a fer realitat que l'estada fos a París, per ser tan bo, per incloure'm a tants plans i no deixar-me mai sola; gràcies per tot!

Fora de la uni, a molts us he d'agrair tot el que feu dia a dia per mi. Als amics de sempre, que m'heu fet costat, que m'heu ajudat a esbargir-me en moments més complicats, que vau ser amb mi quan us necessitava a París. Per mil excursions, aventures, viatges i la vostra amistat. Gràcies a tots, Blanca, Artur, Júlia, Núria i, especialment, Judit, Àlex i Marc també pels dies de gimnàs. Que, encara que moltes vegades em queixi i em vulgui escaquejar, veure-us tant sovint, els moments de cotxe i fer una mica d'esport acompanyada em fa molt feliç. A la Gemmix, amb tu vaig començar en aquest món de la ciència però també compartint tantes coses... Gràcies per aquests anys i per, tot i viure lluny, ser tan propera i bona. A la Velia, o la meva Vèlia com m'agrada dir, gràcies per ser la meva amiga Peruana-Catalana més bonica i sempre preocupar-te i recolzar-me. A l'Abel, gràcies per ser el millor i per tot el suport que sempre m'has donat, sense tu no seria el mateix. Us estimo molt!

A la meva família per ser la millor del món. Gràcies a tiets, tietes i als cosins per ser com sou i estimar-me tant. Gràcies papa per sempre voler el millor per mi i pels teus consells. Gràcies àvia per, juntament amb l'avi, ser el meu referent a la vida, per cuidar-me sempre i per fer-me més fàcils els dies. A l'Eduard, per treure'm de polleguera i ser al meu costat cada dia. I a la mama, qui ho ha fet sempre tot per nosaltres. Gràcies per cuidar-nos cada dia, per deixar-me preparat el dinar cada matí, per enviar-me menjar fins i tot quan era a París, per cada dia animar-me i ajudar-me en tot, gràcies per fer la súper portada d'aquesta tesi. Ets la millor, mameta. Us estimo molt a tots.

The consumption of insects is gaining attention due to its perceived sustainability and health benefits. Compared to traditional protein sources, insect farming is more environmentally friendly, utilising organic waste, emitting fewer greenhouse gases, and requiring less water. Moreover, insects provide a high nutritional value, including essential amino acids and micronutrients, while also containing bioactive compounds like peptides and antioxidants. These compounds show promise in reducing chronic disease risk, improving immune function, and promoting intestinal health. Nevertheless, further research is required to elucidate the health implications of insect consumption and to enhance social acceptance. In this sense, the intestinal tract's health is vital for overall well-being, as it serves as the body's first barrier against food and pathogens. Additionally, the presence of taste receptors in the intestine, which are known to have physiological effects beyond their role in taste perception, highlights the potential for dietary choices to influence intestinal and overall health through these receptors.

In this context, the current thesis examines the impact of insect consumption on intestinal health and, subsequently, on systemic responses, in both healthy and induced inflammation conditions in rats. Furthermore, it investigates the potential modulation of intestinal taste receptors by insects and its health implications.

The results demonstrate that the administration of *Tenebrio molitor* and *Alphitobius diaperinus* to normal chow-feed Wistar rats have no adverse health effects. In fact, supplementation with insects improve alterations in uraemia, glycaemia, and intestinal surface area induced by LPS injection. Insect supplementation modulates umami and bitter taste receptor expression, primarily in the colon, and it has implications for metabolic and inflammatory responses. On the other hand, consumption of *Alphitobius diaperinus*, as a unique source of protein in the diet, resulted in a less pro-inflammatory profile compared to conventional protein sources and induced changes in metabolic status in healthy rats. Additionally, consumption of this insect-based diet attenuates some alterations associated with an obesogenic status, ameliorating inflammatory and metabolic disturbances.

In conclusion, the present thesis demonstrates favourable effects of insect consumption intestinal health as well as inflammatory and metabolic amelioration when insects are consumed chronically in both healthy and pathological conditions. Furthermore, the results reinforce the idea that the expression of intestinal taste receptors is highly susceptible to changes in dietary patterns and health status and suggest its involvement in several important functions including immune and metabolic processes.

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