Circular feeding strategies for *Hermetia illucens* larvae (Diptera: Stratiomyidae):

Effects on performance, bioconversion efficiency, and body composition

Laura Schneider

Inaugural-Dissertation zur Erlangung des Grades eines
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“All creatures great and small.”
– C. F. Alexander, 1848

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Abbreviations

a	Antenna
AA	Amino acids
BSFL	Black soldier fly larvae
BSE	Bovine Spongiforme Enzephalopathie
clf	Clypeofrontal region
CP	Crude protein
DOL	Day-old-larvae
DM	Dry matter
EAA	Essential amino acids
EC	European Commission
Eph	Epipharynx
E	Eye
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization of the United Nations
FM	Fresh matter
FCR	Feed conversion ratio
g	Gena
GE	Gross energy
GECR	Gross energy conversion ratio
gp	Triangular genal projection
Gt CO_2eq	Gigatonnes of Carbon Dioxide Equivalent
IPIFF	International Platform of Insects for Food and Feed
HF	House fly
HVAC	Heating, Ventilation, and Air Conditioning
Lb	Labium
lbr	Labrum
mdr	Mandibular ridges
mxb	Maxillary brush
mxp	Maxillary palp
mxr	Maxillary ridge
N	Nitrogen
NCR	Nitrogen conversion ratio

NDFom	Neutral detergent fiber organic matter
OECD	Organisation for Economic Co-operation and Development
PAPs	Processed animal proteins
P+C	Protein and carbohydrate content
P:C	Protein-to-carbohydrate ratio
SDG	Sustainable Development Goals
TAACR	Total amino acid conversion ratio
Vp	Ventral plate

1 General Introduction

The global demand for sustainable protein sources is rising, posing challenges for livestock production, food security, and environmental protection. Conventional protein feeds, such as soybean and fish meal, face economic, ecological, and ethical limitations, creating a need for alternative, high-quality proteins for animal nutrition (OECD/FAO, 2019; van Huis, 2020). Biowaste and agro-industrial by-products offer potential as circular bioeconomy resources, being convertible into protein- and lipid-rich biomass while mitigating environmental impacts (Rossi et al., 2025; Makkar et al., 2014; van Huis et al., 2013). Insects, particularly the black soldier fly larvae (BSFL, *Hermetia illucens*), are highly efficient at converting organic residues into nutrient-dense feed ingredients.

BSFL transform low-value substrates into biomass suitable for poultry, pigs, aquaculture, and pets (Gasco et al., 2023; Barragán-Fonseca et al., 2017; Gold et al., 2020a). Their nutrient profile, including essential amino acids, fatty acids, and minerals, supports digestibility and performance comparable to or exceeding conventional protein sources (De Marco et al., 2015; Schiavone et al., 2017). Insect-based feeds additionally contribute to sustainability by reducing environmental impacts relative to traditional feeds (Smetana et al., 2023). Larval growth, development, nutrient utilisation, and body composition are strongly influenced by dietary macronutrient composition, substrate quality, and environmental factors such as temperature, rearing density, and microbial load (Oonincx and Finke, 2021; Spranghers et al., 2017; Yakti et al., 2023).

The aim of this thesis was to investigate how dietary composition, substrate characteristics, and feeding strategies affect *Hermetia illucens* larval growth, nutrient utilization, and body composition under controlled and scalable conditions. Three studies were conducted: (Chapter 3) a screening study to establish cultivation standards, (Chapter 4) an evaluation of dietary protein levels in isoenergetic diets and their effects on nitrogen and amino acid retention, and (Chapter 5) an assessment of dietary protein levels in isoenergetic diets on larval performance and nutrient composition across development.The first chapter summarizes the current state of knowledge regarding sustainable protein sources, biowaste valorization, insect nutrition, and the potential of BSFL for feed applications. The subsequent chapters present the peer-reviewed publications of this doctoral thesis, with the final chapter integrating the findings, discussing their implications for BSFL production, and providing perspectives for future research to optimise larval nutrition, enhance biomass quality, and support sustainable insect-based feed production.

2 General context

2.1 The growing need for sustainable protein sources

Global population growth, coupled with rising incomes and dietary shifts, is placing pressure on global food systems. The world population, estimated at 7.8 billion in 2019 (FAOSTAT, 2019), is expected to reach 9.7 billion by 2050 (FAO, 2018). At the same time, economic expansion in regions such as East and Southeast Asia is accelerating the transition toward more animal-based diets (OECD/FAO, 2019). This growing demand for livestock products intensifies competition for high-quality protein resources, particularly because a considerable share of protein used in animal feed originates from crops that are equally suitable for direct human consumption (Mottet et al., 2017; Guillaume, 2025).

In recent decades, global per capita consumption of animal protein has increased significantly—from 9 grams per person per day in 1961 to 20 grams in 2011, with projections of 22 grams by 2030 and 25 grams by 2050 (FAO, 2017, Guillaume, 2025). This trend, as shown in Figure 2.1, highlights the urgent need to develop sustainable production systems that can meet this demand without further harming the environment.

Currently, soybean meal and fishmeal are the primary protein sources for livestock and aquaculture feeds (FAO, 2022; Olsen and Hasan, 2012). Both, however, face significant sustainability challenges. Soybean production is associated with substantial environmental burdens, including extensive land and water use, long-distance transportation, and deforestation, particularly in regions such as the Amazon basin (Hecht, 2005; Guillaume, 2025). Although fishmeal provides a high-quality protein source for animal nutrition, its availability is constrained by the overexploitation of marine resources and pronounced price volatility (Van Huis et al., 2013; OECD/FAO, 2019). Moreover, global protein production is unevenly distributed, creating structural dependencies in several regions. The European Union, for example, imports approximately one quarter of its feed protein supply, while domestic self-sufficiency in soybean meal remains as low as 3% (EC, 2019). Such reliance on external markets heightens vulnerability to geopolitical and economic disruptions and poses challenges to long-term food and feed security (Clément et al., 2018).

Animal protein production further faces the challenge of high environmental costs associated with livestock farming, which contributes approximately 12% of global anthropogenic greenhouse gas emissions, mainly from enteric methane and manure management (FAO, 2023). Feed conversion ratios add to this inefficiency: poultry production requires about 3.0 kg of feed protein to produce 1 kg of edible protein, pigs need 4.3 kg, and ruminants require between 8.3 and over 30 kg (Wilkinson, 2011). These inefficiencies, coupled with the significant land-use demands of animal agriculture, underscore the urgent need for alternative protein sources.

Aquaculture illustrates both the potential and the challenges of animal protein production. Fish contribute approximately 18% of the global supply of animal protein and provide essential nutrients like fatty acids (FAO, 2017). However, capture fisheries have stagnated since the 1990s due to stock depletion, while aquaculture has grown annually by 6.7% since 1990, reaching 87.5 million tonnes (live weight equivalent) in 2020 and is projected to exceed 100 million tonnes by 2027 (FAO, 2022, Guillaume, 2025). Nevertheless, aquaculture remains heavily reliant on fishmeal and soybean meal, raising sustainability concerns. Soybean production, concentrated in South America, is linked to deforestation and greenhouse gas emissions (Jia et al., 2020), while around 20% of capture fisheries are directed to fishmeal and fish oil production, exposing the sector to ecological and climatic uncertainties like the El Niño–Southern Oscillation (FAO, 2022).

In this context, developing novel, sustainable protein sources is imperative. Insects have emerged as a particularly promising solution. Their production requires relatively few resources, generates low environmental impacts, and allows the conversion of low-value organic substrates into high-quality protein (Dicke, 2018; IPIFF, 2018). Black soldier fly larvae (*Hermetia illucens*, BSFL) and the housefly (*Musca domestica*, HF) are of particular interest, as they can be efficiently reared on a wide variety of organic by-products while providing nutritionally valuable feed ingredients (Dörper et al., 2021; Bava et al., 2019; Jucker et al., 2019; Miranda et al., 2020). Utilizing such alternative protein sources could alleviate feed–food competition, enhance sustainability, and contribute to the objectives of the United Nations Sustainable Development Goals (SDG), particularly SDG 2 'Zero Hunger' and SDG 12 'Responsible Consumption and Production' (FAO et al., 2023).

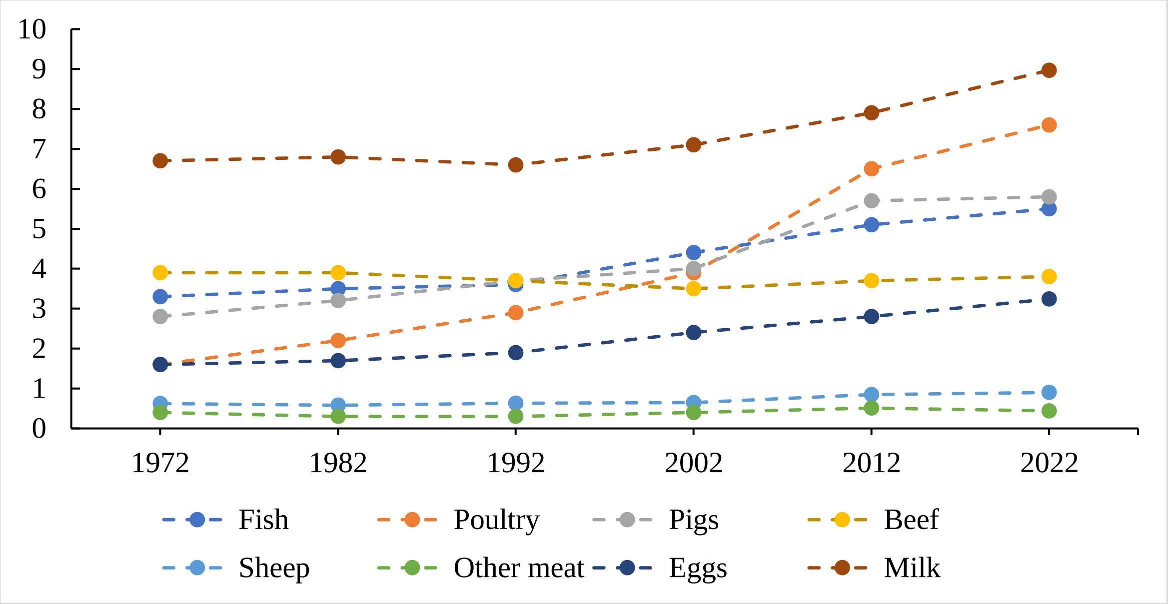

Figure 2.1: Evolution of the average per capita protein intake by source. Modified from FAO, 2024

2.2 Global challenges in biowaste management and agro-industrial by-products

Alongside the rising demand for sustainable feed proteins, the world faces the significant challenge of managing vast quantities of organic waste. Current estimates suggest that over 1.3 billion tonnes of food waste are generated annually, representing about one-third of all food produced for human consumption (FAO, 2015). Additionally, agro-industrial processing yields considerable amounts of nutrient-rich by-products, such as fruit pomace, oilseed cakes, distillers' grains, and crop residues, much of which remains underutilized. This paradox of simultaneous food waste and widespread food insecurity underscores a fundamental inefficiency in the global food system (Papargyropoulou et al., 2014).

Food loss and waste represent missed opportunities for nutrient recovery and pose significant environmental burdens. Food waste releases about 4.4 gigatonnes of CO_2 equivalent greenhouse gases into the air each year, which is about 8% of all human-made emissions. If food waste were a country, it would rank as the third-largest emitter after China and the United States (FAO, 2015). Additionally, food waste exacerbates land-use conflicts, water depletion, and energy demand, while improper management fosters pathogen proliferation and pollution (Ziraba et al., 2016; Omolayo et al., 2021; Guillaume, 2025).

The European Union has responded with policy frameworks such as the Waste Framework Directive (EC, 2025), which establishes a hierarchy of prevention, reuse, recycling, and recovery, prioritizing these over landfilling and incineration. Regulation (EU) 2017/893 authorises insect-derived proteins in aquaculture, pig, and poultry feed, while maintaining strict bans on high-risk substrates such as catering waste. However, conventional waste management remains resource-inefficient: landfilling drives methane emissions and groundwater risks, composting recovers nutrients only to a limited extent, and incineration results in irreversible nutrient losses and additional environmental burdens.

Against this backdrop, the bioconversion of organic side streams through insects has emerged as a promising strategy within the circular economy paradigm (Rossi et al., 2025). Specifically, Hermetia illucens larvae can efficiently transform heterogeneous waste streams into valuable protein and lipid fractions, offering both a sustainable feed source and an environmentally sound method of waste reduction (Gold et al., 2020a). This dual capability positions insect bioconversion as a cornerstone innovation capable of addressing both the protein gap in animal nutrition and the global challenge of biowaste management.

2.3 Insects in circular food and feed systems

The integration of insects into livestock diets is increasingly viewed as a strategic pathway toward more sustainable animal production, reflected in the expanding scientific literature (Van Huis, 2020). Their high nutrient density, potential health-promoting effects, and capacity to upcycle biowaste into valuable protein underpin this growing interest (Rossi et al., 2025; Van Huis et al., 2013). Since the FAO’s 2013 report highlighted their systemic relevance for global food and feed security, research has comprehensively characterised their nutritional composition and functional properties across species (Bukkens, 1997; Finke, 2004; Payne et al., 2016; Raubenheimer and Rothman, 2013; Rumpold and Schluter, 2013a; Dörper et al., 2021; Guillaume, 2025)

While insects were first used in livestock diets in 1919 (Linder, 1919), systematic research emerged in the 1960s and 1970s (Calvert et al., 1969; Hale, 1973; Teotia and Miller, 1973, 1974; Ueckert et al., 1972), and recent studies have focused on safety and feeding trials, consolidating insects as a viable alternative protein source (Beller et al., 2024; Gasco et al., 2019; Henry et al., 2015; Makkar et al., 2014; Riddick, 2014; Rumpold and Schluter, 2013b; Sanchez-Muros et al., 2014, 2016).

The nutritional composition of insects varies greatly depending on species, developmental stage, feeding substrate, and environmental conditions. Nevertheless, insects generally represent a rich source of protein (25–75% dry matter) and lipids (10–70% dry matter), as illustrated for yellow mealworm, black soldier fly, and house fly larvae in Table 2.1 (Dörper et al., 2021; Barker et al., 1998; Bukkens, 1997; Finke, 2002, 2013; Oonincx and Dierenfeld, 2012; Rumpold and Schlüter, 2013a). Processing methods such as defatting can further increase the protein content of insect products, reaching levels comparable to conventional feed ingredients like soybean meal (45–60% dry matter) or fishmeal (60–75% dry matter) (Bussler et al., 2016; Heuzé et al., 2015, 2020).

Insects also possess a well-balanced amino acid profile suitable for monogastric species, being particularly rich in essential amino acids like methionine, cysteine, and lysine, although concentrations are generally lower than in fishmeal; representative amino acid patterns of commonly reared insect species are presented in Table 2.2 (Veldkamp and Vernooij, 2021; Oonincx and Finke, 2021; Makkar et al., 2014). Protein digestibility is generally high, although it can be influenced by processing, the proportion of cuticular proteins complexed with chitin, and mineral content (Rodriguez-Rodriguez et al., 2022; Finke, 2007; DeMarco et al., 2015).

Protein content is commonly estimated by multiplying total nitrogen by a protein factor (Kp = 6.25), resulting in crude protein content (Oonincx and Finke, 2021). However, this method may overestimate true protein content due to non-protein nitrogen sources like chitin or uric acid, while methodological issues can also lead to underestimation (Janssen et al., 2017). Based on amino acid composition, an insect-specific Kp of 4.76–5.81 has been suggested, although retaining 6.25 facilitates comparisons across studies (Dörper et al., 2021; Finke, 2002, 2007, 2013, Janssen et al., 2017).

Amino acid profiles are generally stable within life stages of holometabolous insects but vary between larvae and adults due to morphological differences (Finke, 2002; Pieterse and Pretorius, 2014). Insects’ amino acid profiles are largely unaffected by diet, although the first limiting amino acid varies depending on the consuming species, with methionine and cysteine commonly limiting for monogastric animals (Dörper et al., 2021). Protein and amino acid digestibility in insects are often comparable to conventional feed ingredients, although variability exists depending on species, processing, and analytical methods (Bosch et al., 2014, 2016; Megido et al., 2018).

Insect lipid content is highly variable (10–70% of dry matter) and includes glycerides, sterols, waxes, and fat-soluble vitamins (Bukkens, 1997; Finke, 2013; Yang et al., 2006). Fatty acid composition is influenced by species, life stage, sex, diet, and environmental conditions (Oonincx and Finke, 2021; Finke and Oonincx, 2017; Kulma et al., 2019). Commercially reared insects typically have higher fat content than wild-caught species, often reflecting high-energy diets and reduced activity. BSFL are rich in saturated fatty acids, yellow mealworm larvae contain predominantly unsaturated fatty acids, and house crickets exhibit a balanced fatty acid profile (Oonincx and Finke, 2021; Finke, 2013; Oonincx et al., 2015a; Spranghers et al., 2017). Essential fatty acids, such as linoleic and linolenic acids, cannot be synthesized by most insects, though exceptions exist in certain species (Borgeson and Blomquist, 1993; Borgeson et al., 1991).

Carbohydrate content in insects is generally low (<7% dry matter) and often reflects residual substrate in the gut rather than true tissue composition (Barker et al., 1998; Finke, 2002; Ramos-Elorduy et al., 2002).

Insects contain significant amounts of fiber, including sclerotized proteins, minerals, and chitin, which is a N-acetyl-β-D-glucosamine polymer contributing to cuticle rigidity (Finke, 2007; Kramer et al., 1995; Moussian, 2010). Fiber content varies with species and life stage, with harder-bodied insects typically containing higher amounts of sclerotized protein and acid detergent fiber. Many animal species possess chitinases capable of degrading chitin, although in vivo digestibility remains poorly characterized (Fujimoto et al., 2002; Paoletti et al., 2007).

A wide range of macro-minerals (Ca, P, Mg, Na, K, Cl) and trace minerals (Fe, Zn, Cu, Mn, I, Se) is present in insects, with concentrations typically sufficient to meet the dietary requirements of most animals (Barker et al., 1998; Finke, 2002, 2013; Oonincx and Finke, 2021; Oonincx and Dierenfeld, 2012). Calcium is typically low (<0.3% dry matter) due to the lack of a mineralized skeleton, except in species such as black soldier fly larvae and face fly larvae with mineralized cuticles. Phosphorus is usually present in higher amounts and is bioavailable (Dashefsky et al., 1976). Mineral content can vary due to diet and species-specific characteristics, while anti-nutritional factors such as phytates, oxalates, and tannins are generally low in insects (Ekpo, 2011; Omotoso, 2006).

Insects contain vitamins A, D, E, and B-complex in amounts highly dependent on species and diet (Rumpold and Schlüter, 2013). Vitamin A in insects is largely restricted to the compound eye, while carotenoids in the diet can serve as precursors. Vitamin D3 can be synthesized de novo upon UV-B exposure in some species, such as yellow mealworm larvae, but not in black soldier fly larvae (Oonincx et al., 2018; Oonincx and Finke, 2021). Vitamin E and B-vitamins are generally present in sufficient amounts, although levels can be reduced by processing (Oonincx and Finke, 2021). Thiamine content is highly variable, and some species contain thiaminases that degrade thiamine unless inactivated by heat (Nishimune et al., 2000; Ehigie et al., 2013). Insects also provide choline (1.57–7.26 mg/kg dry matter) and, to a lesser extent, taurine, particularly in adult stages (Finke, 2002, 2013, 2015a, b). Sterols, primarily derived from diet, serve structural and physiological functions, including precursors for molting hormones and vitamin D (Jing and Behmer, 2020).

Table 2.1: Proximate composition (minimum and maximum) of yellow mealworm, black soldier fly, and house fly larvae modified from Dörper et al., 2021

	Dry matter (DM) g/kg fresh	Crude protein g/kg DM	Crude fat g/kg DM	References
Yellow mealworm	364-424	494-661	245-360	Azagoh et al., 2016; Elahi et al., 2019; Ghaly and Alkoaik, 2009; Ochoa Sanabria et al., 2019
Black soldier fly	300-388	371-492	72-387	Finke, 2013; Jucker et al., 2020; Schmitt et al., 2019; Star et al., 2020; Veldkamp and van Niekerk, 2019; Woods et al., 2020
House fly	205-276	579-646	156-245	Fitches et al., 2019; Qi et al., 2019; Wang et al., 2013

Table 2.2: Amino acid patterns (mg/g crude protein) of four commonly raised insects for various species modified from Oonincx and Finke, 2021

Amino acid	*Acheta domesticus* adults/nymphs	*Tenebrio molitor* larvae	*Zophobas morio* larvae	*Hermetia illucens* larvae/prepupae
Alanine	87.8	80.2	72.7	62.7
Arginine	65.7	60.0	57.4	52.8
Aspartic acid	79.1	81.0	83.1	88.3
Glutamic acid	109.2	112.1	127.0	103.7
Glycine	52.3	53.1	48.7	55.0
Histidine	22.8	30.2	31.1	32.4
Isoleucine	40.3	46.1	46.9	43.3
Leucine	78.6	84.9	80.4	69.9
Lysine	55.3	55.4	54.6	59.1
Methionine	15.8	13.3	12.2	18.8
Methionine + cystine	24.9	23.3	21.8	24.4
Phenylalanine	31.5	35.2	37.2	41.5
Phenylalanine + tyrosine	87.5	102.6	108.4	112.3
Proline	56.7	68.1	55.9	55.4
Serine	44.1	47.2	44.1	38.3
Threonine	35.7	40.3	39.9	39.0
Tryptophan	7.2	10.5	11.4	15.1
Valine	54.1	62.9	60.5	63.8

The amino acid data shown for insects are an average of data from published sources (Finke, 2002, 2007, 2015b; Spranghers et al., 2017).

2.3.1 Insects in livestock nutrition and circular feed systems

The favourable nutritional profile of insects has accelerated their exploration as food and feed ingredients (Oonincx and Finke, 2021; Guillaume, 2025). Approximately 2,100 edible species have been identified worldwide, and regular consumption is estimated for hundreds of millions to two billion people, particularly in Asia, sub-Saharan Africa, and Latin America (van Huis, 2022; van Huis et al., 2022). While insects were traditionally harvested from the wild, concerns regarding seasonal variability, contamination risks (e.g., pesticides, heavy metals, mycotoxins), habitat degradation, and overexploitation have increasingly shifted production toward controlled rearing systems (van Huis and Oonincx, 2017, Guillaume, 2025).

In Western countries, entomophagy remains constrained by social resistance, regulatory requirements such as Novel Food authorization in the EU, and comparatively high production costs (Ribeiro et al., 2023; Lähteenmäki-Uutela et al., 2021; Zuk-Golaszewska et al., 2022). By contrast, the use of insects in animal feed is more broadly accepted and increasingly regulated (Bunker and Zscheischler, 2023; Jeinsen and Weinrich, 2023; Oonincx and Finke, 2021; Guillaume, 2025). In the European Union, processed insect proteins from selected species have been authorized for aquaculture and pet feed since 2017 and for poultry and pigs since 2021, while remaining prohibited in ruminant diets (Regulations (EU) 2017/893; 2021/1372). Approved species include *Hermetia illucens*, *Musca domestica*, *Tenebrio molitor*, *Alphitobius diaperinus*, *Acheta domesticus*, *Gryllodes sigillatus*, *Gryllus assimilis*, and *Bombyx mori* (Regulation (EU) 2021/1925). In addition, insect-derived fats and hydrolysed proteins are permitted for pets and several livestock categories (IPIFF, 2022; Lähteenmäki-Uutela et al., 2021; Guillaume, 2025).

In aquaculture, insects constitute a context-dependent alternative to conventional protein sources, with performance determined by insect species, processing strategy, inclusion rate, and target species (Gasco et al., 2023; Hua, 2021). Inclusion levels exceeding 25–30% frequently constrain growth or protein digestibility (Liland et al., 2021), indicating biological and nutritional limits to direct substitution. Optimizing amino acid and fatty acid balance through species blending may partially overcome these constraints (Nogales-Mérida et al., 2018; Guillaume, 2025). Importantly, substitution effects depend on the reference protein: meta-analytical evidence shows that replacing fishmeal with BSFL meal can reduce growth in salmonids, whereas replacing plant-based proteins may enhance growth performance and feed efficiency (Weththasinghe et al., 2021). In crustaceans, even high levels of fishmeal replacement appear feasible without negative zootechnical consequences (Röthig et al., 2023; Guillaume, 2025), underscoring the species-specific nature of optimal inclusion strategies.

In poultry nutrition, insect meals have been associated with improved growth performance and product quality in both broilers and laying hens (Dörper et al., 2021; Sajid et al., 2023; Beller et al., 2024). However, performance responses appear dose-dependent, as inclusion levels exceeding ~10% may compromise growth (Moula and Detilleux, 2019; Salahuddin et al., 2024), Defatted BSF meal at up to 10% inclusion increased feed intake and average daily gain during early growth, while higher levels reduced growth and caecal bacterial diversity (Dabbou et al., 2018; Biasato et al., 2020). Inclusion of 6–8% BSF meal improved feed conversion ratio (FCR) without affecting final body weight (Attivi et al., 2020). Housefly

(HF) larval meal at low inclusion (<2%) increased final body weight and body weight gain, with higher feed intake and improved FCR observed at 0.8–2% inclusion (Okah and Onwujiariri, 2012). Inclusion up to 22% HF meal did not affect nutrient retention or performance (Adeniji, 2007). Amino acid digestibility in HF larvae is comparable to fishmeal (Hall et al., 2018). Variability across studies may result from differences in crude fat content, feed color, and larval processing (Elahi et al., 2019; Elwert et al., 2010). Partially defatted BSF meal improved carcass traits such as breast yield (Dörper et al., 2021; Schiavone et al., 2019). HF larval meal increased dressing percentage and maintained or improved sensory qualities (Dörper et al., 2021). Fatty acid composition of broiler meat reflected larval fatty acid profiles, which depend on the rearing substrate (Cullere et al., 2019; Schiavone et al., 2019).

In pigs, available data remain limited but suggest that insect meals can partially replace soybean meal or fishmeal without compromising performance; however, inclusion levels above ~10% may negatively affect growth (Gasco et al., 2023; Veldkamp and Vernooij, 2021). In nursing piglets, 3.5% BSF meal in creep diets had no effect on feed intake or weight gain (Driemeyer, 2016). In weaned piglets, TM and BSF meal generally did not negatively affect growth, while TM larvae sometimes increased feed intake and body weight gain (Jin et al., 2016; Meyer et al., 2020; Ao and Kim, 2019; Biasato et al., 2019; Spranghers et al., 2018). BSF oil supplementation up to 6% improved growth and FCR (Van Heugten et al., 2019). In growing and finishing pigs, partial or full replacement of fishmeal by BSF or HF larvae generally maintained or improved performance. Inclusion of 4% BSF larvae improved final body weight and FCR, while 8% inclusion showed no benefit, possibly due to higher chitin content (Yu et al., 2020).

Research on insects as ruminant feed remains scarce due to legislative and psychological barriers following the BSE outbreak in the late 1990s, as insect products may potentially act as prion vectors (Renna et al., 2022; Guillaume, 2025). Insect meals are generally less digestible than plant meals, though insect fats may help mitigate ruminal methane emissions (Renna et al., 2023, Guillaume, 2025).

In companion animals, insect meals show nitrogen digestibility comparable to conventional ingredients, making them suitable for pet food (Bosch and Swanson, 2021). Claims of hypoallergenicity are frequent but currently lack strong evidence (Siddiqui et al., 2023).

Beyond their nutritional value, insects provide bioactive compounds—including short-chain fatty acids (e.g., lauric acid), chitin, and antimicrobial peptides—that can enhance immunity, modulate gut microbiota, and serve as alternatives to antibiotics (Biasato et al., 2023; Gasco et al., 2021; Guillaume, 2025). Live insects also offer behavioral enrichment, stimulating natural foraging and pecking activities in poultry and pigs (Stöhr et al., 2025; Ipema et al., 2021, 2022; Schiavone and Castillo, 2023; Rossi et al., 2025).

Despite their potential, legal and safety constraints limit insect use in animal feed. Current EU regulations primarily allow live insects, with broader authorization for commercial poultry production expected (IPIFF, 2018). Feed safety is critical, as larval substrates derived from waste streams may carry chemical or biological contaminants, and only vegetable materials are generally permitted (Van Huis, 2020; IPIFF, 2019). Sustainable production therefore requires approaches that avoid competition with human or conventional animal feed, such as valorizing plant-processing by-products like brewery residues for insect rearing (Guillaume, 2025; Bava et al., 2019).

2.3.2 Circular economy

Beyond their nutritional and health benefits, insects are increasingly recognized as a sustainable feed source due to their ability to upcycle organic waste into high-quality biomass (Rossi et al., 2025; Guillaume, 2025). Many species efficiently utilize agricultural by-products, while saprophagous Diptera larvae—such as black soldier fly and housefly—can convert diverse food waste and other organic residues into valuable nutrients, supporting circular economy strategies by closing resource loops and reducing waste disposal needs (Bai et al., 2023; Parry et al., 2021; Patón and García-Gómez, 2023). The residual material from insect rearing, known as frass, is rich in minerals, chitin, and beneficial microbial communities, which can enhance soil fertility, plant growth, and stress resilience (Gold et al., 2022; Chavez and Uchanski, 2021; Poveda, 2021). By integrating insect production with existing agricultural and food-processing systems, it is possible to recover nutrients from by-products that would otherwise be lost, simultaneously contributing to sustainable feed supply and environmental mitigation.

While insects can efficiently upcycle organic waste, rearing them on such substrates raises microbiological and chemical safety concerns (Vandeweyer et al., 2021; Lievens et al., 2021; Malematja et al., 2023; Guillaume, 2025). To ensure feed safety and maintain circular economy standards, the EU permits only approved substrates—including plant materials, processed animal by-products, and pre-consumer food residues—while post-consumer waste, meat-based residues, and feces remain prohibited (Regulation (EC) No 1069/2009). By contrast, regulatory frameworks in many Asian, African, and South American countries are less restrictive, offering greater flexibility for substrate selection (Ojha et al., 2020; Purkayastha and Sarkar, 2022).

Insects demonstrate high feed conversion efficiency, converting up to 26% of dry matter and 87% of protein into body mass, surpassing conventional livestock (Nakagaki and Defoliart, 1991; Oonincx et al., 2015; Wilkinson, 2011). Their poikilothermy and low maintenance energy requirements underpin this efficiency, while controlled production systems can further optimize nutrient recovery and resource use, reinforcing the circularity and sustainability of insect-based feed.

Life cycle assessments highlight strong sustainability potential: insect production typically emits fewer greenhouse gases and requires less land than beef or pork, though performance may vary relative to poultry or fish (Smetana et al., 2023; Vauterin et al., 2021). Defatted BSFL meal (2.5-5.3 kg CO_2-eq/kg) falls within the range of soybean and fishmeal, and using post-consumer waste can even lead to net negative emissions (Smetana et al., 2021). Renewable energy use and industrial waste heat further improve environmental outcomes, and scaling and genetic improvement are expected to enhance efficiency (Vesterlund et al., 2024; Eriksson and Picard, 2021).

2.3.3 *Hermetia illucens*

The black soldier fly (BSF), *Hermetia illucens*, a Diptera of the Stratiomyidae family, is the most widely farmed insect in the food and feed sector (Guillaume, 2025). While it occurs naturally in tropical to temperate regions worldwide (Maquart et al., 2020), genomic evidence indicates a South American origin (Kaya et al., 2021; Marshall et al., 2015). Historical records, including a 16th-century larval find in an Italian sarcophagus, suggest introduction to Europe via transatlantic shipping (Benelli et al., 2014; Guillaume, 2025). Initially regarded as a pest causing myiasis and later studied in forensic entomology, BSF became of interest for waste management and animal feed in the late 20th century (Liu et al., 2023; Tomberlin and van Huis, 2020).

BSF undergoes complete metamorphosis (holometaboly), progressing through four main stages as illustrated in Figure 2.2 (Guillaume, 2025; Bellezza Oddon et al., 2022a; Cammack and Tomberlin, 2017):

- **Eggs:** Elongated, white to yellowish, <1 mm, ~25 µg, laid in clutches of 400–800 in sheltered sites near decomposing material; incubation ~4 days (Dortmans et al., 2021; Meneguz et al., 2022).
- **Larvae:** Six instars, feeding actively on semi-liquid organic matter and accumulating protein and fat for subsequent stages. Early instars are off-white to cream, while prepupae darken, increase exploratory behavior, and search for a pupation site. Larval development typically lasts 15–23 days, with prepupae reaching 12–25 mm and 120–250 mg (Kim et al., 2010; Caruso et al., 2014).
- **Pupa:** Immobile and non-feeding, pupae metabolize stored fat to develop adult structures, including wings, legs, compound eyes, and reproductive organs. Pupal duration is 10–15 days.
- **Adult (imago):** 12–20 mm, black with translucent abdominal segment and smoky wings, sponging mouthparts for liquid intake only. Adults display complex mating behavior including lekking and in-flight copulation and live 5–8 days, extendable to ~1 month if liquid feed is available (Julita et al., 2020; Lemke et al., 2022; Bruno et al., 2019; Lupi et al., 2019).

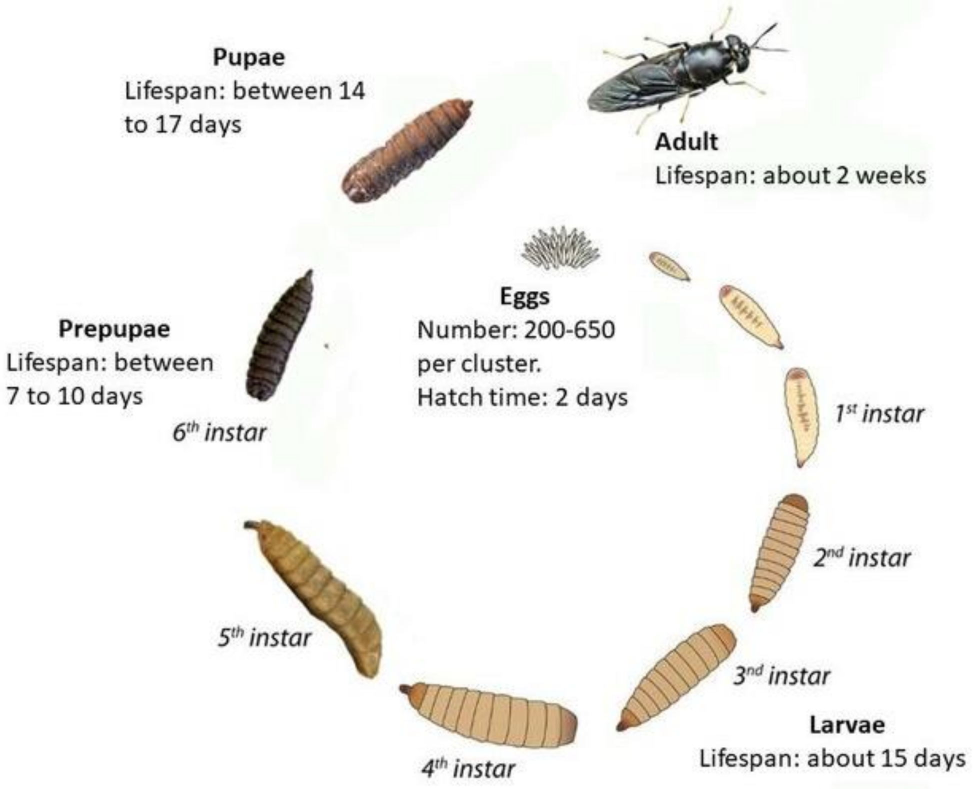

Figure 2.2: Life cycle of the black soldier fly (Hermetia illucens), with average duration of each development stage. From Maquart (2019).

BSF is widely used for biowaste conversion and feed production due to its rapid development, broad substrate utilization, and remarkable larval growth (up to 10,000-fold within two weeks). BSF is considered safe for humans, animals, and plants, and its cosmopolitan distribution minimizes invasive-species risks. Larvae remain within the substrate, enabling rearing in open, stackable trays, while adults exhibit limited flight and are easily contained.

Industrial production relies on controlled environmental conditions. Adults mate and oviposit in aviaries; eggs are collected and incubated; neonates are applied to nursery trays and subsequently transferred to rearing units. Larvae are harvested after about one week, separated from frass, and processed into protein meal, oil, and fertilizer. Increasing automation (e.g., conveyors, robotics, computer vision) improves efficiency across breeding, rearing, and processing stages. Other BSFL production models exist, including decentralized facilities separating breeding, rearing, and processing (Guillaume, 2025). Less capital-intensive approaches are common in the Global South, taking advantage of favorable climates and sometimes relying on spontaneous oviposition from local wild populations. Diener and Gold (2022) identified at least 125 BSF companies worldwide. The sector has diversified, with some players focusing on starter larvae, equipment (e.g., crates, machinery, heating, ventilation and air conditioning (HVAC) systems), or services (e.g., engineering, advisory, software) (Guillaume, 2025).

Designing a BSFL rearing operation requires identifying suitable feedstuffs to ensure optimal larval growth and reproduction, high-quality end-products, compliance with animal welfare standards, and positive environmental and economic outcomes. Achieving these objectives requires a precise understanding of BSFL nutrition and conducting feed trials to determine the best substrates.

2.4 Current knowledge on the nutrition of *Hermetia illucens*

2.4.1 Organ systems regulating nutrient processing

BSFL are capable of developing on a remarkably wide variety of substrates, which differ in nutrient content and environmental characteristics. Their ability to thrive under such variable conditions is rooted in specialized physiological adaptations that regulate digestion, nutrient absorption, and metabolism (Pinotti and Ottoboni, 2021; Guillaume, 2025). BSFL also display coordinated social feeding behavior: larvae move collectively in fountain-like patterns, which enhances access to nutrients and increases overall feeding efficiency (Shishkov et al., 2019). Their conical-shaped head and robust mouthparts are specifically adapted for semi-liquid substrates (Figure 2.3). Larvae use their mandibular-maxillary apparatus to separate coarse particles from smaller ones, which are then ground and ingested. The mouthparts have been compared to a tunnel-boring machine (Bruno et al., 2020; Kim et al., 2010). The maximal particle size ingested ranges from 20 to 150 µm, depending on larval instar (Lievens et al., 2023; Liland et al., 2017).

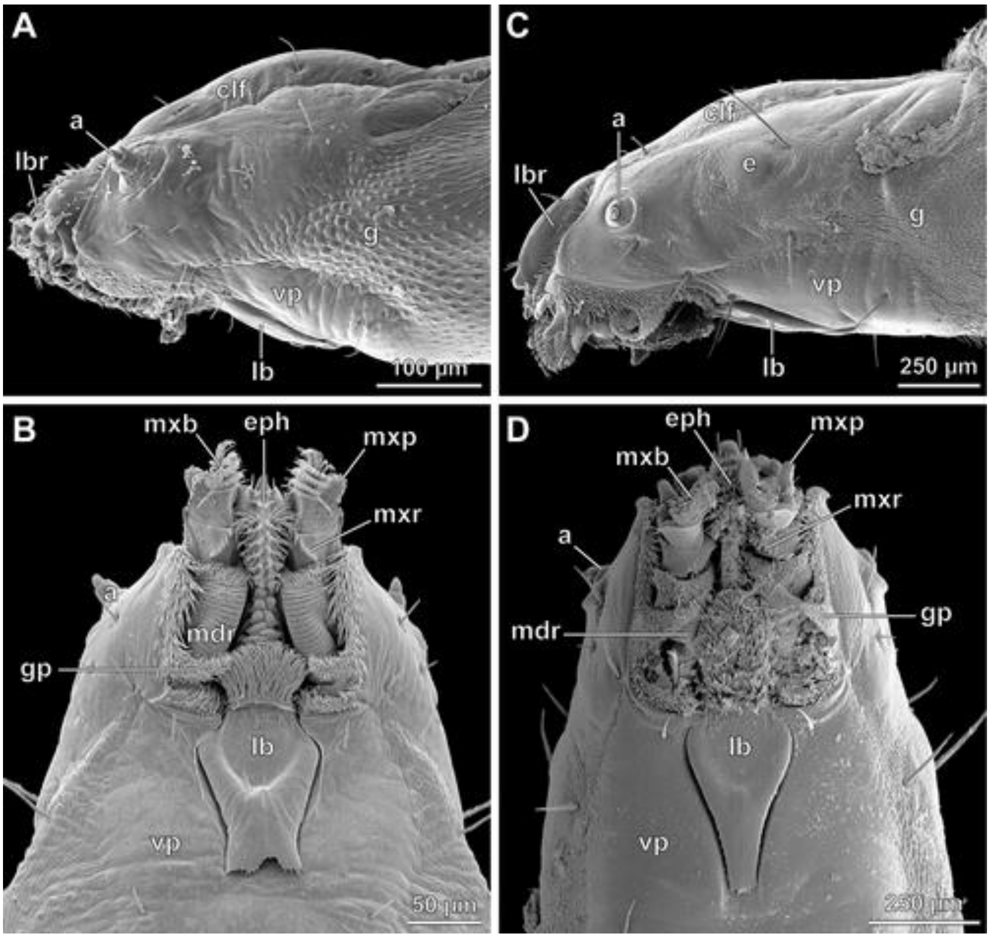

Figure 2.3: Hermetia illucens, head of 3rd and penultimate instar, SEM images. (A) 3rd instar, lateral view. (B) 3rd instar, ventral view. (C) penultimate instar, lateral view. (D) penultimate instar, ventral view. Abbreviations: a – antenna, clf – clypeofrontal region, e – eye, eph – epipharynx, g – gena, gp – triangular genal projection, lb – labium, lbr – labrum, mdr – mandibular ridges, mxb – maxillary brush, mxp – maxillary palp, mxr – maxillary ridge, vp – ventral plate. From Fabian et al. (2025).

Like all insects, the BSFL digestive tract is divided into three sections (Figure 2.4):

Foregut (stomodeum): Extending from the mouth to the proventriculus, the foregut is involved in ingestion, grinding, storage, and transport of food to the midgut (Gullan and Cranston, 2014).

Midgut (mesenteron): The midgut is the primary site for enzymatic digestion and nutrient absorption and is subdivided into three regions, each with distinct functions, cell types, and associated microbes (Caccia et al., 2019; Vandeweyer et al., 2023). The anterior midgut (pH 6.0) secretes amylase and lipase, the median midgut is highly acidic (pH 2.0) and digests microorganisms via lysozyme, and the posterior midgut (pH 8.5) exhibits peptidase and lipase activity (Bonelli et al., 2019). Morphological, transcriptomic, and microbial features of the midgut are highly plastic, adapting to different feeding substrates (Bonelli et al., 2020; Bruno et al., 2018; Sun et al., 2021). The peritrophic membrane, a semipermeable structure of chitin, protein, and carbohydrates, separates the midgut lumen into endo- and ecto-peritrophic spaces. This arrangement increases digestive efficiency, protects the midgut epithelium, and allows enzyme recirculation (Lin et al., 2021). Using dyed substrates, total food residence time in the midgut was measured at less than four hours (Gold et al., 2020b).

Hindgut (proctodeum): Responsible for water and ion balance, excretion of faeces and urine, the hindgut starts with Malpighian tubules. These structures absorb nitrogenous waste, water, ions, amino acids, and sugars from the haemolymph and release them into the ileum, where urine and undigested food mix. The rectum then reabsorbs water, salts, amino acids, and sugars before excretion (Gullan and Cranston, 2014; O'Donnell and Donini, 2017).

Absorbed nutrients are distributed via the haemolymph to all organs. The fat body, a whitish structure throughout the haemocoel, serves as the main site for nutrient storage and metabolism. Sugars are converted into lipids (lipogenesis) and stored as intracellular lipid droplets, while glycogen and protein granules provide additional reserves (Hoc et al., 2020; Pimentel et al., 2017; Roma et al., 2010; Zhu et al., 2019). Storage and mobilization of these reserves are regulated by nutritional and hormonal signals, including adipokinetic hormones from the brain (Li et al., 2019; Skowronek et al., 2021). The fat body also plays a central role in reproductive development, such as oogenesis (Arrese and Soulages, 2010).

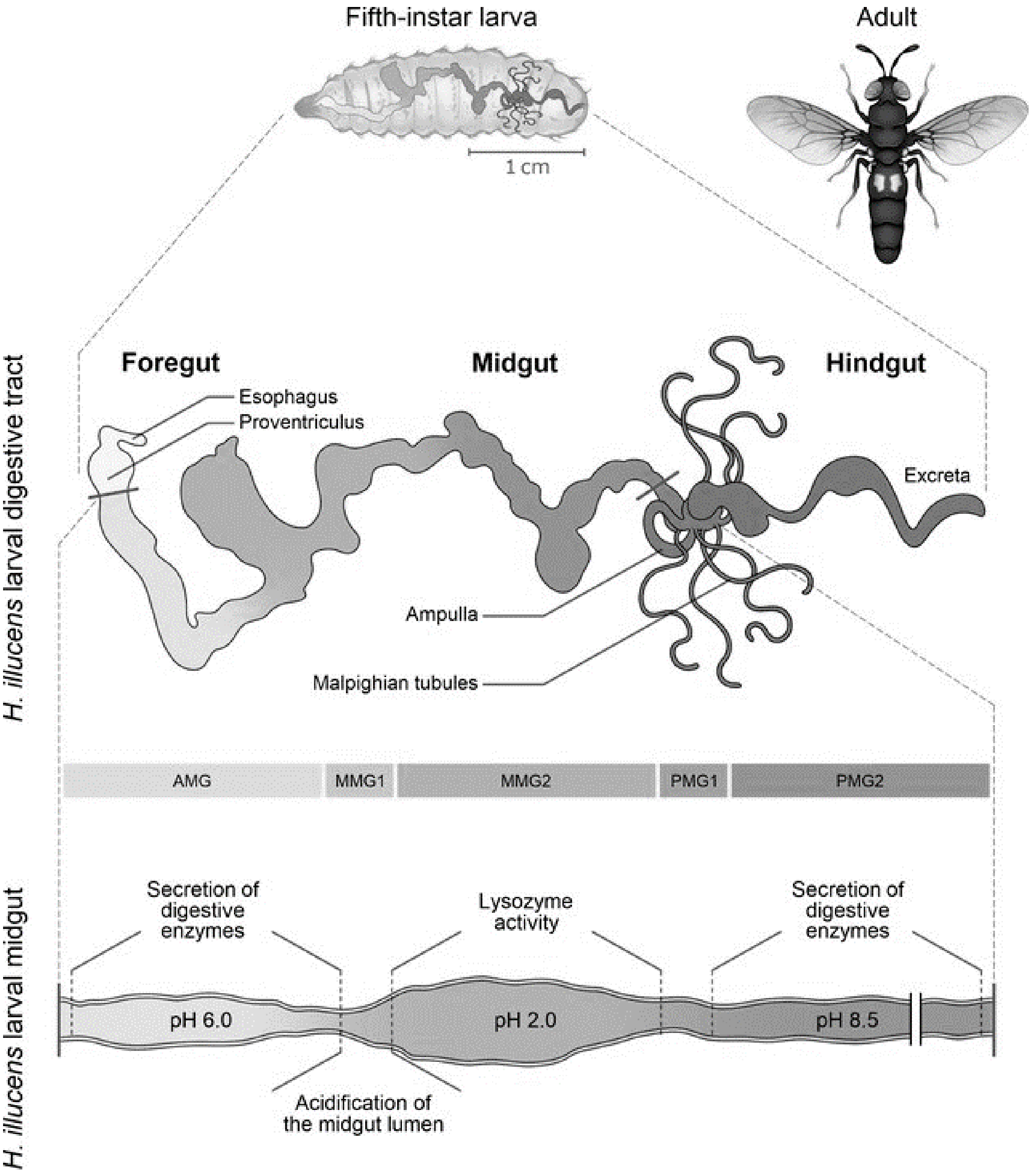

Figure 2.4: Schematic representation of the digestive tract of H. illucens larvae. The digestive tract of this species is characterized by an exceptionally long midgut, which is divided into distinct regions with specific physico-(bio)chemical conditions and digestive function. Bonelli et al. (2019) demonstrated that each midgut region hosts unique bacterial communities, indicating a compartmentalization of microbial partners in accordance with the digestive processes. AMG, anterior midgut; MMG, middle midgut; PMG, posterior midgut. Modified from Eke et al., 2023

Despite this knowledge, several aspects of BSFL nutrition remain poorly understood. Key questions include the detailed processes of food ingestion, mechanisms of gut plasticity, larva–microbe interactions, factors affecting digestive efficiency, and metabolic regulation pathways (Tettamanti et al., 2022). Advancing our understanding of these processes promises to improve BSFL feed conversion efficiency and optimize their use in animal feed production.

2.4.2 Measuring black soldier fly performance in feed trials

Several life history traits and performance indicators are used to evaluate BSF in relation to a given feeding substrate, with the ultimate goal of identifying an optimal diet. While most indicators focus on the larval stage, measurements can also be made on pupae, adults, or eggs laid by flies that were reared on the tested substrate, which offers more information about the suitability of a feed (Guillaume, 2025). Entomologists who studied crop pests came up with these metrics in the past (Waldbauer, 1968; van Loon, 1991; Parra et al., 2012).

Unlike many pest species such as Lepidoptera or Coleoptera larvae, BSFL live within their substrate: they feed, excrete, and hide in it simultaneously. Consequently, the mixture of feed and excreta makes direct measurement of ingested feed or excreta impossible. Therefore, indicators such as efficiency of conversion of ingested or digested feed and consumption rate should be avoided for BSFL studies. Instead, bioconversion efficiency corrected for residue and substrate reduction indices are recommended (Bosch et al., 2020). A notable challenge in the field is the lack of standardisation: terminology is inconsistent (e.g., “growth rate” has been applied to different metrics), and definitions regarding trial duration or the use of fresh versus dry weight are often unclear. Recent efforts emphasize the importance of harmonization and provide guidelines for robust and reproducible BSF feeding trials (Bosch et al., 2020; Deruytter et al., 2023; Nayak et al., 2023).

Each performance indicator captures a different aspect of BSF response to a substrate. For instance, fresh individual larval weight reflects the match between larval growth requirements and substrate quality, but it does not provide information on conversion efficiency relative to time (growth rate) or feed quantity (feed conversion ratio, FCR). Substrates may rank differently depending on whether growth (total larval weight gain), reproduction or waste management (waste reduction) is considered. Hence, the definition of an “optimal diet” must specify the intended goal and the indicator used.

From an economic perspective, substrates may be low-cost, free, or even negatively priced if they constitute waste that a company or municipality seeks to dispose of (e.g., former foodstuffs, municipal biowaste). Conversely, agricultural by-products may be more expensive due to competition with other industries (e.g., conventional livestock feed, biogas production). Among BSFL outputs, insect meal generally commands a higher market price than oil or frass. Accordingly, an economic feed conversion ratio can be calculated by weighting inputs and outputs based on their market value on a dry weight basis. Similarly, environmental feed conversion efficiency can be estimated by combining substrate-specific FCR with environmental footprint data (e.g., global warming potential, land use), since feed is the main contributor to the environmental impact of BSFL production (Smetana et al., 2023).

2.4.3 Substrate as insect feed for larval bio-mass production

A central factor determining productivity in any animal production system is the quality and availability of feed biomass. In the rapidly expanding insect farming sector, however, knowledge about species-specific nutritional requirements remains limited, as large-scale production systems are still relatively recent developments (Pinotti and Ottoboni, 2021).

Unlike conventional livestock systems, the concept of “feed” in insect production is not clearly standardized. In traditional animal nutrition, more than 900 agricultural products are used in feed formulation (FAO, 2013), typically classified as roughage, concentrates, feed supplements, and feed additives (Pinotti and Ottoboni, 2021). Such categories are not directly transferable to insect farming. This is largely due to the biological capacity of insects—particularly species such as *Hermetia illucens*—to valorize substrates of low nutritional quality, including agro-industrial co-products and vegetable residues (Dicke, 2018; Pinotti et al., 2019). Consequently, a broad spectrum of organic materials can serve as nutrient sources for insect rearing, with selection primarily driven by availability, regulatory constraints, technical feasibility within specific production systems, and economic considerations (Pinotti and Ottoboni, 2021).

From a regulatory perspective, insects reared for the production of processed animal proteins (PAPs) are classified as farmed animals under European legislation (EC, 2009b). As a result, they are subject to feed ban provisions (EC, 2001) and general animal feeding regulations (EC, 2009b). This regulatory framework closely links insect production to PAP legislation, albeit in a reversed functional context: insects may serve as a protein source but are simultaneously restricted in the substrates they can be fed (EC, 2017; Ottoboni et al., 2017).

In insect farming, the term “substrate” is commonly used instead of “feed” to describe the materials provided for larval growth. The legal status of substrates remains a dynamic and region-specific issue. Broadly, substrates can be divided into those permitted and those prohibited, with the boundary between categories differing across jurisdictions (Pinotti and Ottoboni, 2021).

Within the European Union, the use of certain materials—such as ruminant-derived proteins, catering waste, meat-and-bone meal, manure, intestinal contents, and sewage sludge—is prohibited, primarily due to regulations addressing transmissible spongiform encephalopathies and bovine spongiform encephalopathy (EC, 2009a). In its risk assessment, EFSA (2015) classified potential substrates into several categories, ranging from conventional feed materials and former foodstuffs to slaughterhouse by-products, catering waste, manure, and sewage sludge. Not all of these categories are authorized for insect feeding (Pinotti and Ottoboni, 2021).

Currently, insect production systems in Western countries predominantly rely on commercial compound feed, plant-based co-products from food processing, and former foodstuffs that do not contain meat or fish (e.g., production surpluses or products past their best-before date but compliant with food legislation) (Giromini et al., 2017; Luciano et al., 2020; Ottoboni et al., 2019; Pinotti et al., 2019; Pinotti and Ottoboni, 2021). In contrast, in several non-European regions—particularly in parts of Africa—a wider array of bio-waste streams is utilized, including animal manure. These materials are typically pre-treated, for example by composting or heat processing, before being used as insect substrates (Münke-Svendsen et al., 2017).

Beyond agro-industrial by-products, insect farmers frequently rely on conventionally formulated feed mixtures composed of cereals and legumes that are seasonally available, including maize, sorghum, and soybean. In addition, various locally grown leafy vegetables are incorporated into rearing systems, such as collard greens, jute mallow, amaranth leaves, black nightshade, cowpea leaves, and spider plant (Pinotti and Ottoboni, 2021).

In regions characterized by seasonal fluctuations, producers may substitute vegetables with locally accessible forage leaves during dry periods. Examples include cassava, banana, sweet potato, tomato, papaya, and moringa leaves (Münke-Svendsen et al., 2017). However, these resources often compete with other agricultural uses and may also serve as human food, creating potential trade-offs between insect farming and food security (Münke-Svendsen et al., 2017).

The choice of substrate is guided by multiple performance-related criteria. Producers consider nutrient composition, expected larval growth and biomass yield, feed conversion efficiency, time required to reach harvestable stages, and reliability of year-round availability. From a product quality perspective, decisive parameters include crude protein content and amino acid profile, fatty acid composition, micronutrient levels (e.g., minerals), absence of contaminants, and practical aspects such as ease of harvesting (Pinotti and Ottoboni, 2021).

Moisture content represents an additional critical factor affecting both productivity and biomass quality. Experimental evidence indicates that increasing substrate moisture levels can negatively influence larval performance (Guillaume, 2025). For instance, trials comparing moisture contents between 40% and 80% demonstrated reductions in feed reduction efficiency, wet larval weight, body size, and developmental performance at higher moisture levels (Dzepe et al., 2020; Salomone et al., 2017). Similarly, excessive water addition was shown to decrease biomass conversion efficiency and larval survival (Lalander et al., 2020). Substrates containing 80–90% moisture require careful management, including adequate ventilation, whereas materials exceeding 90% moisture are generally unsuitable for BSF composting systems, even under active aeration. These findings are particularly relevant for waste management strategies aiming to valorize fruit and vegetable residues through larval bioconversion (Pinotti and Ottoboni, 2021).

Research on BSFL nutrition and growth is expanding rapidly, with dozens of new studies published each month by both academic and industrial groups worldwide. To synthesise this growing body of knowledge, numerous reviews and meta-analyses have been conducted, including those by Gold et al. (2018), Surendra et al. (2020), Pinotti and Ottoboni (2021), Hopkins et al. (2021), and Siddiqui et al. (2022). In these studies, feeding substrates are typically classified into broad categories, such as animal or human faeces, slaughterhouse by-products, restaurant food waste, fruit and vegetable waste, cereal by-products, and compound diets (Guillaume, 2025). Compound diets include commercially formulated feeds like chicken feed or the Gainesville diet, developed by Hogsette (1992) with 50% wheat bran, 30% alfalfa meal, and 20% maize meal, which is commonly used as a reference substrate.

Substrate type has a strong influence on virtually all performance indicators, although substantial variability exists within each category. For instance, slaughterhouse by-products often result in lower larval survival compared with other substrates (Barragan-Fonseca et al., 2017; Hosseindoust et al., 2023). Development time is generally shorter on compound diets than on manure, while straw-based substrates produce the longest development periods

(Fitriana et al., 2022; Gold et al., 2018) (Figure 2.5). Recent reviews indicate that fresh larval weight is higher on compound diets compared with manure or food waste (Fitriana et al., 2022; Hosseindoust et al., 2023), and dry larval weight is maximised on compound diets relative to other substrates, with the lowest dry weight observed on straw-based diets (Fitriana et al., 2022; Pinotti and Ottoboni, 2021). Algae-based diets tend to yield low larval biomass, likely due to antinutritional factors (Pinotti and Ottoboni, 2021).

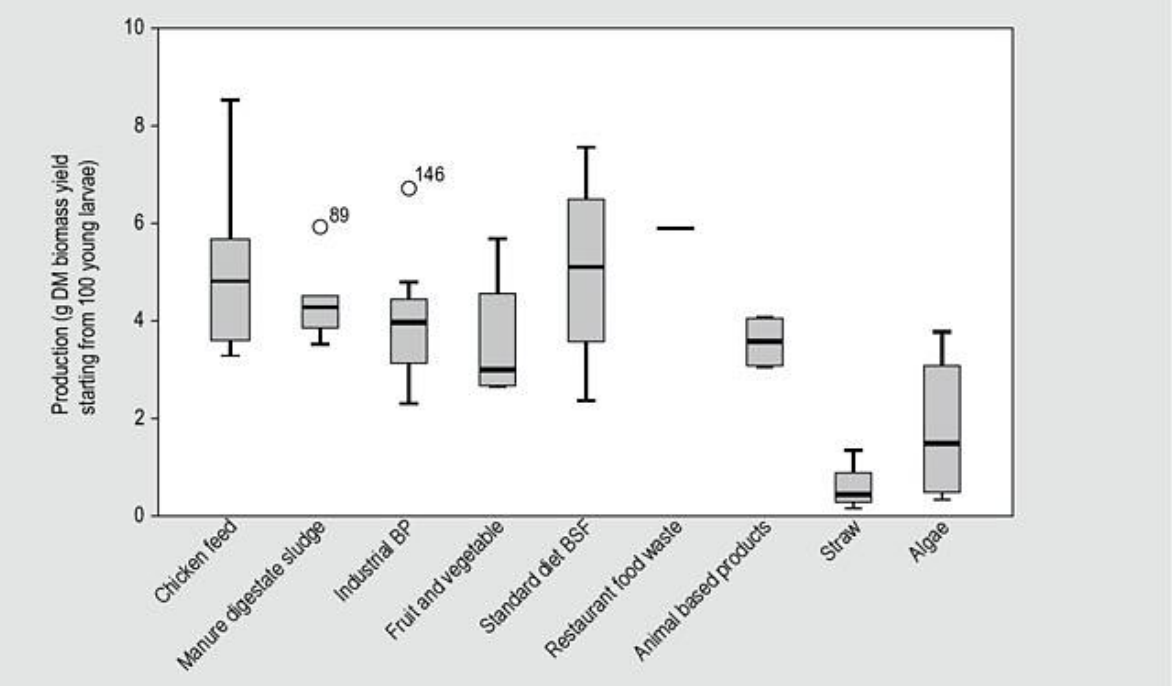

Figure 2.5: Mean, median, quartiles, minimum and maximum values, as well as outliers, illustrating the distribution of larval biomass yield (calculated as grams of larval biomass produced per 100 initial larvae). DM, dry matter. Modified from Pinotti and Ottoboni (2021).

Most reviews report no consistent effect of substrate type on larval nutritional composition, except for Hosseindoust et al. (2023), who observed higher dry matter and protein in larvae fed compound diets and higher lipid content in larvae fed food waste (Pinotti and Ottoboni, 2021). Protein content in BSFL ranges from 12.9% DM on plant-based substrates to 78.8% DM when fed *Sardinella aurita* fish waste (Hopkins et al., 2021). The non-protein fraction of dry matter mainly consists of fat, chitin and minerals, with protein–fat trade-off strongly driven by substrate quality. Amino acid and mineral profiles are generally unaffected by substrate type, while fatty acid composition is influenced by the substrate; for example, inclusion of fish offal or seaweed increases omega-3 fatty acids in BSFL (Cattaneo et al., 2023; Gold et al., 2018).

In conclusion, BSFL can thrive on a wide range of organic substrates, reflecting a highly plastic digestive system. Life history traits tend to improve on mixed or compound diets, presumably due to better nutritional balance. Consequently, reviews recommend focusing on the nutritional profile of substrates rather than on their ingredient composition alone. The need for standardised methodologies in BSFL research is also emphasised (Gold et al., 2018; Hopkins et al., 2021; Pinotti and Ottoboni, 2021).

2.4.4 Dietary determinants of black soldier fly larvae growth and efficiency

To investigate how specific nutrients influence BSFL performance, controlled diet systems are commonly employed. Larvae can be provided with purified diets, containing chemically defined nutrients, semipurified diets, combining purified nutrients with natural ingredients, or diets based solely on raw ingredients with analyzed nutrient content (oligidic diets) (Guillaume, 2025; Bellezza Oddon et al., 2022a). These approaches allow detailed assessment of how individual nutrients or nutrient groups affect larval growth, feed conversion, and biomass composition.

Most studies to date have used oligidic diets, which approximate natural substrates while retaining some level of complexity (Guillaume, 2025). However, there remains a significant gap in insect nutrition research: few studies systematically explore purified or semipurified diets to dissect nutrient-specific effects, limiting our understanding of optimal dietary requirements for BSFL (Bellezza Oddon et al., 2022a; Hosseindoust et al., 2023). This knowledge gap constrains precise formulation of artificial diets for large-scale production.

While such approaches are useful for identifying general trends, they are limited by methodological heterogeneity (e.g., differences in larval density, age at sampling, or protein quantification methods) and potential confounding factors, which can lead to overgeneralised conclusions—particularly when extrapolating results from plant-based substrates to animal by-products.

Protein content of the feeding substrate is consistently positively associated with larval fresh and dry weight (Barragan-Fonseca et al., 2021; Hosseindoust et al., 2023). Iso-energetic diets indicate that 14–16% DM protein maximises larval fresh weight (Bellezza Oddon et al., 2022a). Excessive protein (>20% DM) can impair survival and extend development time, likely due to the metabolic cost of excreting nitrogenous waste (Barragan-Fonseca et al., 2021; Cheon et al., 2021). Lower protein contents (10–12% DM) minimise development time and enhance adult emergence (Barragan-Fonseca et al., 2021). Substrate protein also influences larval protein and fat content, though its effect on protein can vary depending on the analysis framework (Eggink et al., 2023; Barragan-Fonseca et al., 2021). Overall, protein content between 10–15% DM represents a practical compromise for larval survival, growth, and nutritional quality (Guillaume, 2025).

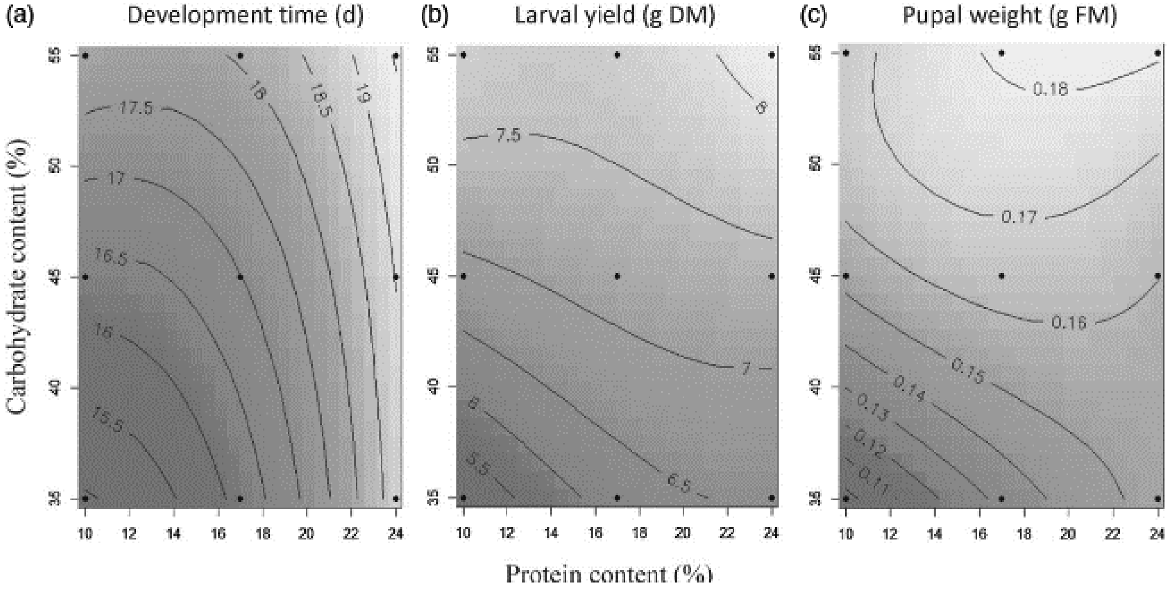

Figure 2.6: Response surfaces showing black soldier fly larval performance on diets with varying protein and carbohydrate concentrations. (a) Development time (days); (b) larval yield per container [g DM]; and (c) individual pupal weight [g FM]. Fitness landscapes illustrate responses across nutrient space. Black dots represent the nine experimental diets. From Barragan-Fonseca et al. (2021).

Amino acid composition of the feeding substrates is another key factor. To date, only lysine requirements are well established for BSFL (~0.3% DM for optimal growth), while the needs for tryptophan, threonine, methionine, and other amino acids are under investigation (Guillaume, 2025; Koethe et al., 2021; Moradei et al., 2023). The amino acid profile of BSFL remains relatively stable and is generally independent of substrate composition, mirroring patterns observed in conventional livestock (Fitriana et al., 2022; Hopkins et al., 2021).

Carbohydrate content also strongly affects larval growth and performance. Substrates containing 40–50% DM non-fibre carbohydrates optimise fresh and dry larval weight, whereas excessive carbohydrate levels reduce survival (Barragan-Fonseca et al., 2021; Cheon et al., 2021; Hosseindoust et al., 2023). High-carbohydrate diets increase larval fat content and improve adult performance (lifespan, individual weight, total egg weight), although they slightly reduce larval protein content (Guillaume, 2025). Protein and carbohydrate recommendations are often summarised using total macronutrient content (P+C) and protein-to-carbohydrate ratio (P:C). Optimal performance is generally observed with P+C between 25–50% DM and P:C ratios of 1:2–1:4 (Barragan-Fonseca et al., 2021; Eggink et al., 2023), though slightly more protein-biased ratios (1:1.1–1:1.8) have been suggested depending on specific performance indicators (Cheon et al., 2021).

Fibre-rich substrates reduce BSFL growth and nutritional quality, likely due to the absence of appropriate digestive enzymes (Gold et al., 2018; Hosseindoust et al., 2023).

Lipid content of the substrate is typically below 20% DM (Gold et al., 2018). Extremely low lipid content (<3.5% DM) impairs larval growth and FCR, suggesting a requirement for essential fatty acids (Bellezza Oddon et al., 2022b; Kieβling et al., 2023). Lipid-rich substrates increase larval weight and fat content but can decrease protein and ash contents, and overly greasy substrates may hinder feeding due to stickiness (Hosseindoust et al., 2023; Kieβling et al., 2023). Optimal lipid content for larval performance is estimated at 5–10% DM. The fatty acid composition of the substrate is strongly reflected in larval lipids, allowing manipulation of larval omega-3 content through dietary inclusion of fish offal or seaweed (Cattaneo et al., 2023; Hoc et al., 2021; Oonincx et al., 2020). Sterols, which insects cannot synthesise, are essential for structural and hormonal functions, but their requirements in BSFL remain unknown (Jing and Behmer, 2020, Pinotti and Ottoboni, 2021).

Vitamins and minerals in BSFL nutrition are currently poorly understood, representing a major knowledge gap (Seyedalmoosavi et al., 2022).

2.5 General nutrition principles and diet formulation: insights from conventional livestock species and relevance for black soldier fly larvae

To achieve optimal growth and health of farm animals, an ideal ratio of protein and especially essential amino acids in the feed is necessary to ensure consistent and high-quality products of animal origin. Providing feed that meets the physiological needs of a given species ensures optimal growth, reproduction, immunity, and overall health while avoiding nutrient deficiencies or excesses (Adhikari et al., 2025). Standardized, requirement-based diets allow the efficient conversion of feed into desired outputs such as body mass, milk, eggs, or offspring, while minimizing nutrient waste and associated environmental impacts, for example through reduced nitrogen and phosphorus excretion (Bikker and Jansman, 2023). Moreover, formulating diets that meet the species-specific nutritional requirements enhances economic efficiency, given that feed represents the major input cost in animal production systems, and facilitates the identification of limiting nutrients that may impair performance (Bikker and Jansman, 2023). In conventional livestock, nutrient requirements are determined through controlled feeding trials, factorial models, and metabolic studies such as nitrogen balance and indirect calorimetry, with results formalized in standardized feeding tables to guide diet formulation across life stages and production goals. These principles provide the framework for designing optimized diets that balance macronutrients, micronutrients, and energy to maximize performance, product quality, and sustainability (Zuidhof, 2020).

When translating these principles to novel protein sources such as insects, similar approaches are applied, though they must be adapted to the species' biology and rearing conditions. In BSFL, for example, feed is consumed within a dynamic substrate environment, complicating direct measurement of intake (Guillaume, 2025). Researchers therefore rely on controlled experimental diets and performance indicators to infer nutrient requirements, aiming to maximize larval growth, nutrient retention, and feed conversion efficiency while accounting for environmental and microbial influences (Barragan-Fonseca et al., 2017).

Consequently, requirement studies in BSFL rely on modified experimental approaches and innovative techniques tailored to these biological and ecological characteristics (Bellezza Oddon et al., 2022a).

By monitoring larval performance indicators such as growth rate, individual larval weight, development time, survival, pupation success, adult emergence, and nutrient composition of harvested larvae, researchers can infer the levels of macro- and micronutrients required for optimal development (Pinotti and Ottoboni, 2021). These indicators serve as proxies for the physiological adequacy of the diet, analogous to weight gain or milk production in livestock studies (Oonincx et al., 2015; Barragan-Fonseca et al., 2017).

Dose–response experimental designs, in which larvae are provided with diets varying in protein, carbohydrate, lipid, or amino acid content, are particularly informative. For example, studies manipulating substrate protein levels have identified ranges that maximize larval weight and survival, while excessive or deficient levels can impair development and feed conversion efficiency (Bellezza Oddon et al., 2022b). Similarly, varying carbohydrate or lipid content helps to define optimal energy provision for growth and lipid accumulation, which are essential for pupal development and adult reproductive performance (Oonincx and Finke, 2021). Nutrient balance and ratio approaches, inspired by the nutritional geometry framework used in other animal models, have also been applied to BSFL to explore the interactive effects of multiple nutrients and identify optimal protein-to-carbohydrate ratios for different production objectives (Barragan-Fonseca et al., 2021; Bellezza Oddon et al., 2022a).

Beyond macronutrients, measuring micronutrient requirements—including essential minerals, vitamins, and specific amino acids—remains largely exploratory in BSFL. Preliminary studies on lysine requirements demonstrate the feasibility of identifying critical amino acids using iso-formulated diets, but the comprehensive determination of all essential nutrients is still ongoing (Lemme and Klüber, 2024; Spranghers et al., 2017; Nguyen et al., 2015). Analytical techniques, such as nutrient profiling of both feed substrates and larvae, along with mass balance calculations, allow researchers to assess nutrient assimilation and retention, thereby providing indirect estimates of dietary requirements (Guillaume, 2025).

Environmental and microbial factors must also be considered, as they can modulate nutrient availability and utilization. Microbial pre-digestion of substrates, larval gut microbiota, substrate pH, moisture content, and temperature all influence larval nutrient uptake and growth (Vogel et al., 2022; Yakti et al., 2023). Thus, requirement studies often standardize rearing conditions or include controls to account for these variables, ensuring that observed effects are attributable to diet composition rather than external factors (Bellezza Oddon et al., 2022b).

In conclusion, measuring optimised diets in case of nutrient requirements in BSFL integrates concepts from conventional livestock nutrition—such as controlled feeding trials, dose–response approaches, and performance monitoring—but requires adaptations to account for the unique biological, ecological, and operational characteristics of the species. These studies are essential for defining optimal diets that maximize larval growth, nutrient composition, and feed conversion efficiency, providing a foundation for both scientific understanding and industrial applications in sustainable insect protein production (Oonincx and Finke, 2021; Barragan-Fonseca et al., 2017).

2.6 Objectives

In the context of an increasing global demand for sustainable protein sources, *Hermetia illucens* (black soldier fly larvae, BSFL) have emerged as a promising candidate for converting low-value organic materials into high-value nutrients suitable for animal feed. To optimise the zootechnical performance of BSFL—such as weight gain, protein deposition, and overall nutrient efficiency—a precise understanding of their specific nutritional requirements is essential.

A growing body of research has provided valuable insights into the nutritional physiology of BSFL (Barragan-Fonseca et al., 2021; Bellezza Oddon et al., 2022a; Bellezza Oddon et al., 2022b), offering reference values that can serve as constraints for diet formulation using diverse feed ingredients. However, diet formulations based solely on crude nutrient concentrations have been shown to yield inconsistent larval performance (Bellezza Oddon et al., 2024).

This observation highlights the need for more accurate nutritional models that consider the utilisation of crude nutrient contents of substrates. We hypothesise that formulating BSFL diets based on nutrient contents—defined for each nutrient and ingredient—will ensure a more accurate nutrient supply to the larvae. Consequently, this approach is expected to reduce performance variability and improve both the biological and economic efficiency of BSFL production systems.

The present doctoral thesis was designed to test the following hypotheses:

1. Variations in substrate type influence the growth performance, nutrient utilisation efficiency, and body composition of *Hermetia illucens* larvae.
2. Dietary protein concentration affects the nutrient utilisation efficiency of *Hermetia illucens* larvae.
3. Dietary protein concentration influences enhances growth performance, developmental dynamics, and nutrient composition of *Hermetia illucens* across its life stages.

The individual experiments and their findings are presented in the subsequent chapters. Chapter 3, Chapter 4 and Chapter 5 address the specific effects of dietary composition and nutrient concentration on larval performance and nutrient utilisation, while Chapter 6 integrates these findings within the broader context of circular feeding strategies and sustainable insect production systems.

3 Publication I

Effects of various by-products of the food industry on performance and energy and nitrogen retention in black soldier fly larvae

Authors

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Conflict of interest

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Abstract

The rearing of black soldier fly larvae represents a sustainable approach to organic waste valorization and animal feed production. However, substrate composition is known to influence larval growth, nutrient assimilation, and body composition. This study evaluated the effects of seven food industry by-products—carrot scraps, grape pomace, biscuit meal, bread crumbs, wheat bran, wheat pulp, and rapeseed cake—compared to the standard Gainesville diet under commercial rearing conditions. Larvae were maintained in 144 containers (approximately 14,000 larvae per container) within a climate-controlled room. Each of the eight treatments was replicated 18 times, with six replicates harvested on days 4, 8, and 12 post-hatching for analysis. Substrate type significantly affected growth, survival, and feed conversion, with interactions between substrate and harvest time ($P < 0.05$). Wheat bran yielded the highest survival rate, biomass gain, and feed conversion efficiency, whereas carrot scraps and grape pomace resulted in the lowest values. Nitrogen retention was greatest in larvae fed wheat bran, while wheat pulp and rapeseed cake showed the lowest retention. Biscuit meal and bread crumbs improved energy retention compared to carrot scraps and grape pomace. Frass composition closely reflected substrate nutrient profiles, particularly nitrogen and energy content. Harvest timing also significantly influenced both larval and frass composition. These findings highlight the critical role of substrate selection in optimizing nutrient utilization, growth performance, and biomass yield in BSFL production.

Keywords

Hermetia illucens, substainable feed, insect biomass

1. Introduction

Edible insects are increasingly recognised as a sustainable protein source, aligning with the growing demand for alternative feed and food production (Bessa *et al*., 2020; Van Huis *et al*., 2025; Rossi *et al*., 2025). Among them, black soldier fly larvae (*Hermetia illucens*) excel in converting organic substrates into protein- and fat-rich biomass, making them integral to waste management systems (Gao *et al*., 2019; Liew *et al*., 2023). Due to their high-density production and minimal spatial requirements, BSFL can be efficiently reared on agricultural and food industry side streams, further supporting circular economy principles (Cammack e*t al*., 2017; Fitriana *et al*., 2022; Yakti *et al*., 2023). Insect farming increasingly explores agro-industrial by-products as sustainable substrates. This study focuses on seven side streams selected based on availability in Europe, nutritional value, and potential suitability for rearing *Hermetia illucens* larvae. Carrot pomace (CS) is a fibrous by-product of juice production. It is typically sent to biogas plants for energy recovery, although its nutritional profile suggests alternative uses in animal and insect feed (Mousavi *et al*., 2023). Grape pomace (GP) is the primary residue from winemaking, comprising skins, seeds, and stems. In 2023, global grape production reached approximately 73.5 million tonnes on 6.7 million hectares, predominantly from *Vitis vinifera*. GP accounts for ~85% of total winemaking residues and is mostly discarded or applied as vineyard fertiliser, despite its residual fibre and nutrient content (Lopes *et al*., 2025). Bakery by-products, such as biscuit meal (BM) and bread crumbs (BC), result from overproduction, product returns, and defective goods. The European bakery sector generates around 3.5 million tonnes of such residues annually, with high energy density due to their carbohydrate and residual fat content (Malamakis *et al*., 2023). Variability in composition and perishability limits their broader application. Wheat bran (WB) is a by product of dry milling and represents approximately 2–3% of the total grain weight. In 2023, the EU harvested around 125.9 million tonnes of wheat and spelt making wheat bran a widely available substrate rich in fibre, protein, and minerals (Saini and Sinha, 2024). Wheat pulp (WP), produced during wheat starch extraction, is primarily generated in Germany and the Netherlands. It is rich in digestible fibre and residual protein and is traditionally used in ruminant feeding (FEFAC, 2022). Rapeseed cake (RSC) is the solid fraction left after oil extraction from *Brassica napus* seeds. With ~19.3 million tonnes of rapeseed processed in the EU in 2023/24 (EU Commission, 2025), the resulting protein-rich cake is widely applied in animal nutrition and offers potential for insect farming. The nutritional composition of substrates used for rearing BSFL critically influences performance indicators such as feed conversion ratio (FCR), growth rate, nutrient retention, and overall biomass yield (Taufek *et al*., 2024; Scala *et al*., 2020; Barbi *et al*., 2020; Oonincx *et al*., 2019, Liu *et al*., 2018; Meneguez *et al*., 2018, Nguyen *et al*., 2013). Among the dietary components, the protein content in substrates plays a particularly central role. Previous studies recommend a crude protein content in the range of 14–30% in order to ensure increased larval growth (Bellezza Oddon *et al*., 2022; Barragán-Fonseca *et al*., 2021). Beyond the absolute quantity, the source and digestibility of protein also determine the degree to which dietary nitrogen is retained in the larval biomass (Guillaume *et al*., 2023; Schneider et al., 2025). Studies of Spranghers *et al*. (2016) and Naser El Deen *et al*. (2023) have further demonstrated that the overall nutritional quality of organic side streams has a decisive impact on larval viability, growth rate, and biomass accumulation. Considerable variation in development time, weight gain, and nutrient composition of the larvae has been linked to differences in feed macronutrient profiles, including proteins, carbohydrates, lipids, fibre, and ash (Gold *et al*., 2018; Hopkins *et al*., 2021). In this context, not only the absolute protein content but also the ratio of protein to energy in diets has been identified as a key determinant of life-history traits such as insect development time and larval body size (Barragán-Fonseca *et al*., 2021; Schneider *et al*., 2025) as well as the requirement for aminoacids 98 (Lemme and Klüber, 2024, Tomberlin *et al*., 2023). Likewise, the chemical composition of larval frass is strongly determined by the type of larval feed, particularly its nitrogen and energy contents (Basri *et al*., 2022; Fuhrmann *et al*., 2022). Compared to conventional livestock species, insects require relatively lower amounts of protein, fat, carbohydrates, and water to support growth and development (Oonincx *et al*., 2010). This conversion efficiency makes them ideal candidates for the bioconversion of organic side streams into high-value protein with reduced environmental impact (Van Huis *et al*., 2025; Rossi *et al*., 2025). Consequently, the use of agricultural and food processing side streams as rearing substrates represents a promising strategy to address the global demand for sustainable protein sources. A major challenge in insect farming is the considerable variability in the chemical composition and nutritional quality of agro-industrial by-products, which directly influences nutrient utilisation and larval performance. The extent to which substrate composition affects growth efficiency, nutrient retention, and larval body composition in *Hermetia illucens* remains insufficiently characterised. This study addresses this gap by evaluating seven regionally available food by-products—carrot scraps, grape pomace, biscuit meal, bread crumbs, wheat bran, wheat pulp, and rapeseed cake—as alternative rearing substrates. Under commercial conditions, we assessed their impact on larval growth, nutrient utilization, and biomass composition. Additionally, chemical analyses of frass were conducted to examine substrate related differences in nutrient allocation. These insights advance our understanding of substrate suitability for insect farming, offering valuable perspectives for optimising larval nutrition and improving sustainable protein production in alignment with circular bioeconomy goals (FAO, 2018).

2. Material and methods

2.1 Substrate preparation

Seven agro-industrial side streams were used as rearing substrates: CS, GP, BM, BC, WB, WP, and RSC. All materials were obtained from regional producers in Germany and processed according to standard industrial practices to ensure consistency and preserve nutrient quality. CS, derived from carrot juice production, was obtained from a vegetable processing facility (Gemüse Renner V+V GmbH, Mutterstadt, Germany) and used without further treatment. GP, a coarse mix of skins, seeds, and stems from grape pressing, was obtained from a winery (Weingut Hofmann, Appenheim, Germany) and used without homogenization. BM and BC originated from a bakery (Backhaus Lüning, Bingen, Germany) and consisted of ground biscuits and dried shredded bread products. WB, WP, and RSC were supplied by a feed and grain processor (AGRAVIS Raiffeisen AG, Münster, Germany). A conventional Gainesville diet (CON), consisting of wheat bran, maize (AGRAVIS Raiffeisen AG, Münster, Germany), and alfalfa, served as the control (Hogsette, 1992). Alfalfa, where applicable, was obtained as dried grass from Agrobs GmbH & Co. KG (Degerndorf, Germany) according to the specified formulation. The dry matter content of all substrates was determined using a moisture analyzer (Radwag MA 200/1.X2.IC.A, Radon, Poland). Subsequently, substrates were weighed and mixed with warm tap water (27 °C) to reach a standardized moisture content of 75%. After homogenisation, one representative sample per container (150 g) was collected and analysed for its nutrient composition (Table 3.1). Based on the results of the chemical analyses, the food by-products used in this study are listed below according to their calculated nitrogen-to-gross energy (N:GE) ratio (Table 3.1).

Table 3.1: Nutrient composition of various side streams and control feed

	Substrates[a]							
Nutrient (g/kg)	CS	GP	BM	BC	WB	WP	RSC	CON
Dry matter	751±10.2	748±9.3	739±12.5	745±5.5	751±9.5	754±9.5	753±6.9	752±9.5
Gross energy (MJ/kg)	15.4±0.5	15.5±0.4	18.4±0.1	17.4±0.4	17.1±0.2	19.2±0.6	18.0±0.2	16.6±0.4
Nitrogen	12.5±0.2	12.9±0.3	15.7±0.2	18.1±0.5	24.3±0.6	42.1±0.7	45.7±1.0	24.4±0.7
N:GE ratio (g/MJ GE)	0.82±0.02	0.83±0.02	0.90±0.03	1.04±0.01	1.42±0.01	2.19±0.08	2.52±0.00	1.47±0.04
Crude protein[1]	81±1.1	81±0.6	98±1.4	113±2.8	152±1.8	263±6.9	286±5.9	153±4.3
Crude fiber	129±3.8	213±6.0	18±0.3	7.5±0.2	95±2.4	41±1.1	162±3.4	148±3.1
Ether extract	13±2.7	22±9.9	113±2.8	53±1.3	42±1.5	54±0.8	56±1.4	24±3.5
Starch	20±1.9	17±0.5	403±5.7	512±7.3	147±2.5	31±0.2	69±1.6	213±3.5
Ash	116±3.8	40±1.6	33±0.5	26 ±0.4	63±1.6	66±0.5	71±0.5	63±1.4
Calcium	6.23±0.3	5.21±0.0	1.48±0.1	0.67±0.1	0.96±0.0	2.070.1±	9.01±0.1	6.90±0.3
Phosphorus	3.05±0.1	2.43±0.0	3.17±0.1	3.67±0.2	12.31±0.2	9.07±0.1	10.88±0.1	7.70±0.2
Essential amino acids (g/kg)								
Arginine	3.29±0.05	7.38±0.01	5.04±0.02	5.15±0.07	11.1±0.14	14.0±0.12	18.72±0.12	7.38±0.10
Histidine	1.36±0.06	3.19±0.01	2.12±0.06	2.18±0.10	4.24±0.06	6.80±0.00	8.31±0.08	2.90±0.06
Isoleucine	3.31±0.02	4.73±0.04	3.66±0.03	3.21±0.06	5.11±0.03	11.13±0.07	12.32±0.10	4.82±0.04
Leucine	5.64±0.06	8.14±0.02	6.94±0.04	6.00±0.06	9.92±0.04	21.6±0.06	21.18±0.03	9.63±0.05
Lysine	2.81±0.09	5.12±0.07	3.30±0.06	3.72±0.04	6.52±0.05	6.91±0.14	18.30±0.13	4.42±0.07
Methionine	1.01±0.04	2.04±0.04	1.54±0.02	1.56±0.01	2.38±0.06	5.03±0.08	6.14±0.04	2.01±0.03
Phenylalanine	3.60±0.02	5.03±0.05	4.43±0.05	4.31±0.07	6.25±0.04	14.0±0.12	12.18±0.16	5.68±0.05
Threonine	3.09±0.03	4.49±0.04	3.15±0.06	3.24±0.05	5.26±0.02	10.20±0.06	13.75±0.07	4.82±0.04
Tryptophan	1.00±0.02	1.42±0.02	1.36±0.03	1.24±0.01	3.56±0.04	3.20±0.03	4.74±0.08	2.24±0.05
Valine	4.13±0.05	5.72±0.06	4.55±0.03	4.50±0.02	7.52±0.03	14.20±0.06	15.90±0.12	6.60±0.09

Values are mean of a duplicate analysis and standard deviation (SD)

[a] *The tested substrates consist of carrot scraps (CS), grape pomace (GP), biscuit meal (BM), bread crumbs (BC), wheat bran (WB), Gainesville diet (control diet, CON), wheat pulp (WP) and rapeseed cake (RSC) and are categorized according to the nitrogen to gross energy ratio (N:GE ratio).*

[1] *Calculated as nitrogen × 6.25*

2.2 Rearing conditions and sample collection

Black soldier fly larvae (BSFL) were obtained from a commercial colony maintained by madebymade GmbH (Pegau, Germany), originating from more than 50 generations of continuous rearing. Eggs were collected within a 24-hour laying period, and larvae were initially reared for six days on a pre-starter diet consisting of wheat bran and commercial chicken feed (Legekorn, AGRAVIS Raiffeisen AG, Münster, Germany) in a 1:1 ratio (w/w), adjusted to 65% moisture. Rearing was conducted at 28 °C and 70% relative humidity (RH) in a controlled climate chamber.

Experimental trials were performed in the laboratory of the Department of Animal Nutrition, University of Applied Sciences Bingen. On day 6, larvae were size-standardized using sieves (2.4 mm and 1.0 mm mesh) to remove oversized and undersized individuals. The remaining larvae were homogenized by manual stirring, and three 1 g subsamples were randomly collected and counted to determine mean individual fresh weight (FW; 7.5 ± 0.2 mg). Based on these measurements, 105 g of larvae (approximately 14,000 individuals) were allocated to each experimental container (40 × 60 × 12 cm; 2.1 cm^3 per larva). Eight treatments (seven side-stream substrates and one control diet) were tested in 18 replicates, resulting in 144 containers. Rearing was conducted in a room was controlled climate conditions (27 ± 1.5 °C, 55 ± 5% RH), and larvae were randomly assigned to one of the experimental substrates or the control diet. Each container received 2,800 g dry matter (DM) of substrate (0.2 g DM/larva; ~0.8 g FM/larva), following established standards for feed intake and substrate utilization (Schneider et al., 2025a).

Six replicates per treatment were harvested after 4, 8, and 12 days. By day 12, most larvae had reached the prepupal stage. No additional feeding or substrate mixing occurred during the trial. At each harvest, larvae were manually separated from residual substrate and frass, rinsed, gently dried with paper towels, and weighed. A subsample of 100 larvae per container was weighed (±0.001 g; KERN ADB/ADJADB 200-4, Balingen, Germany) to calculate mean individual body mass and estimate total larval count. Frass was quantified by subtracting the weights of cleaned larvae and the empty container from the total container weight, including frass adhering to container walls, which was homogenized prior to analysis.

All biological materials (larvae, frass, residual substrate) were stored at −18 °C until analysis. Samples were freeze-dried within 36 hours (20 L freeze-dryer, CHRIST, Osterode am

Harz, Germany) and ground to pass a 1 mm sieve (ZM 100, RETSCH GmbH, Haan, Germany) prior to chemical analysis.

2.3 Proximate composition

Collected samples were analyzed according to the methods of the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA). Nitrogen was measured using the method of Dumas (4.1.2). The crude protein (CP) concentration of each substrate was determined by multiplying its total nitrogen content by 6.25. Since non-protein nitrogen can contribute to total nitrogen measurements, larval protein content was further corrected using the formula $CPcor = N \times 4.76$, following the approach described by Janssen et al. (2019). Dry matter, ether extract, crude fiber, and crude ash were analyzed according to methods 3.1, 5.1.1, 6.1.1, and 8.1, respectively. Calcium (Ca) and Phosphorus (P) were determined by optical emission spectrometry with inductively coupled plasma (ICP-OES Quantima; GBC Scientific Equipment Pty. Ltd., Australia) according to method 10.8.2. Samples were analyzed for their GE (Bomb calorimetry IKA C 5000, Staufen, Germany). Starch in the feed was analyzed polarimetrically according to method number VDLUFA 7.2.1. The chitin content in the insect samples was quantified using the method described by Urs et al. (2023), with slight modifications as outlined in Schneider et al. (2025). To account the complexity of the insect samples, 5mg of freeze-dried insect samples were used instead of one mg, and the pellet in the last step of the sample preparation was resuspended in 500 µL of water before it was filtered using a centrifugal filter (PES 3K) (VWR International, Radnor, USA).

2.4 Calculations

To determine larval growth performance, 100 larvae were randomly weighed on day 1 and at the end of the feeding period. Survival rate was expressed as the ratio of final to initial larval counts (Guillaume et al., 2023). Larval biomass gain was calculated as the difference between total biomass at harvest and initial larval mass in dry matter. The feed conversion ratio (FCR) was calculated as the ratio of substrate mass provided to larval biomass at harvest. To evaluate nutrient and energy utilization, the nitrogen conversion ratio (NCR) and the gross energy conversion ratio (GECR) were determined. NCR was defined as the amount of nitrogen supplied (g) per 100 g of larval biomass gain, while GECR indicated the amount of gross energy supplied (MJ) per 100 g of larval biomass gain. These indicators were calculated based on substrate input and larval output, following the methodology of Schneider et al. (2025). The conversion parameters were determined in fresh matter (FM) and dry matter (DM). Conversion parameter in FM means distributed feed in 88% DM divided by larval gain in FM. Conversion parameters in DM were calculated as distributed feed in 88% DM divided by larval gain in DM. Retention, representing the proportion of either GE or N retained in larval biomass, was calculated as the mass of GE or N in larvae divided by the respective mass in the substrate supplied (Equation 1; Seyedalmoosavi et al., 2023).

$$\text{Retention [\% in dry matter]} = \frac{Larval\ gain\ (g)}{Substrate\ supply\ (g)} \times 1000$$

2.5 Statistics

Statistical analysis was performed using IBM SPSS Statistics (Version 29). The dataset included larval fresh weight, survival rate, biomass gain, and conversion ratios as well as chemical analyses from six containers with 14,000 larvae per substrate (n=6; N=144). Normality was assessed using QQ-plots, and homogeneity of variance with Levene's test. Data were analyzed using a general linear model (PROC GLM) with fixed effects of substrates, time, and their interaction (Substrates × Time). Tukey post-hoc tests were used to determine significant differences ($P<0.05$).

3. Results

3.1 Larval performance and conversion of nitrogen and gross energy

Table 2 summarizes larval fresh weight, survival rate, and biomass gain. For all these parameters, significant effects of substrate and rearing time and significant substrate x rime interactions were observed ($P<0.001$).

Larval fresh weight increased progressively with rearing time across all substrates. On day 4, larvae reared on WP, RSC, and CON exhibited the highest individual weights, whereas those fed CS and GP showed the lowest values. By day 8, differences among substrates were less pronounced; larvae fed RSC, CON, and WB reached the greatest weights, while WP-fed larvae were slightly lighter than RSC, WB, and CON. CS- and GP-fed larvae consistently displayed the lowest weights. At the final sampling (day 12), larvae reared on BC, RSC, and CON achieved the highest weights, whereas CS and GP remained the lowest.

Survival rates were highest on day 4 and lowest on day 12, although the magnitude of decline from day to day 12 varied among substrates. On day 4, survival ranged between 85% and 95% for most substrates, except WP, which exhibited a markedly reduced rate. Overall, survival was less affected than larval weight. The steepest decline from day 4 to day 12 occurred in CS and RSC. At day 12, the highest survival rates were observed in larvae fed CON and WB, while CS supported the lowest survival. GP, BM, BC, WP, and RSC showed intermediate values.

Biomass gain over the intervals day 0–4, day 0–8, and day 0–12 were substrate-dependent. During day 0–4, the greatest biomass gains were recorded for RSC and CON, while CS and GP yielded the lowest. In the subsequent intervals (day 0–8 and day 0–12), WB and CON supported the highest biomass gains, whereas CS and GP consistently produced the lowest. Biomass gains for the remaining substrates (BM, BC, WP, RSC) were intermediate.

Table 3.2: Larval fresh weight, survival rate, and larval biomass gain of black soldier fly larvae (Hermetia illucens) fed on different substrates for 4 d, 8 d and 12 d rearing.

Item	Subtrates[1]							
	CS	GP	BM	BC	WB	WP	RSC	CON
Larval fresh weight, mg in fresh matter								
D4[2]	40±6^{d}	33±5^{d}	48±8^{c}	82±9^{b}	69±4^{b}	116±8^{a}	132±8^{a}	97±5^{a}
D8[3]	86±5^{d}	102±13^{c}	137±8^{b}	150±12^{a}	169±7^{a}	137±8^{b}	150±8^{a}	148±8^{a}
D12[4]	126±15^{c}	109±10^{d}	140±15^{b}	167±12^{a}	143±7^{b}	129l±6^{c}	149±10^{a}	153±7^{a}
Survival rate, %								
D4[2]	89.4±2.1^{ab}	85.2±4.8^{b}	87.5±8.6^{ab}	91.6±3.5^{a}	94.6±4.3^{a}	74.7±3.9^{d}	80.7±5.3^{c}	90.0±4.9^{a}
D8[3]	61.9±3.6^{c}	68.2±8.5^{b}	74.8±8.2^{b}	72.7±7.4^{b}	77.7±2.2^{a}	69.8±2.9^{b}	68.5±2.6^{b}	82.1±3.3^{a}
D12[4]	42.6±4.3^{e}	66.5±7.6^{c}	72.4±7.4^{b}	65.2±7.0^{b}	75.5±5.2^{a}	67.3±5.0^{c}	55.3±7.0^{c}	76.2±8.5^{a}
Larval biomass gain, g in dry matter								
D0-D4[5]	98±18^{e}	82±20^{e}	131±11^{d}	226±10^{c}	217±17^{c}	287±16^{b}	337±18^{a}	295±11^{a}
D0-D8[6]	134±22^{e}	221±18^{d}	368±28^{c}	395±22^{c}	507±20^{a}	398±16^{c}	456±20^{b}	536±13^{a}
D0-D12[7]	160±25^{e}	175±29^{e}	412±28^{c}	443±25^{b}	471±18^{a}	395±25^{c}	366±28^{d}	524±24^{a}

[1] Parameters of BSFL reared on eight different substrates categorized by nitrogen-to-gross energy ratio: carrot scraps (CS), grape pomace (GP), biscuit meal (BM), bread crumbs (BC), wheat bran (WB), wheat pulp (WP), rapeseed cake (RSC), and control diet (CON). Data are presented as least square means with standard deviations. Superscripts (a-e) for substrate-specific response denote significant pairwise contrasts. Substrate replicates (n = 48), time point replicates (n = 18).

[2] D4: Harvest at day 4 of rearing;

[3] D8: Harvest at day 8 of rearing;

[4] D12: Harvest at day 12 of rearing

[5] D0-D4: Feeding period 0-4 days;

[6] D0-D8: Feeding period 0-8 days;

[7] D0-D12: Feeding period 0-12 days

Feed conversion efficiency was evaluated based on fresh matter and dry matter intake (Table 3). Significant effects of substrate, time, and their interaction ($P<0.001$) were detected for both measures ($P < 0.001$). For fresh matter-based feed conversion, the lowest FCR values during the first 4 and 8 days were observed for BC, WB, and CON, whereas CS exhibited the highest values. Over the entire 12-day period, BC, WB, and CON maintained the lowest FCR, while CS consistently showed the highest.

For dry matter-based feed conversion, substrate effects were similarly pronounced. During the first 4 days, WP, RSC, and CON achieved the lowest FCR values, whereas CS and GP displayed the highest. At day 8 and day 12, CON and WB supported the most efficient feed conversion, while CS remained the least efficient substrate.

Energy conversion efficiency also varied significantly with substrate, time, and their interaction ($P<0.001$, Table 3). During the first 4 days, energy was most efficiently converted into larval growth on RSC and CON, and least efficiently on GP. By day 8, CON and WB exhibited the highest energy conversion efficiency, while CS and GP were the least efficient. Over the full 12-day period, BM, BC, WB, and CON demonstrated the greatest energy conversion efficiency, whereas CS and GP consistently ranked lowest.

Nitrogen conversion efficiency showed similar trends. Significant effects of substrate, time, and their interaction ($P<0.001$) were observed (Table 3). Nitrogen utilization for larval growth was generally less efficient during the first 4 days compared to later intervals. At day 4, CON supported the most efficient nitrogen conversion, while GP showed the lowest. By day 8, WB achieved the highest nitrogen conversion, followed by BM and BC, whereas CS remained the least efficient. At day 12, CON again exhibited the greatest nitrogen utilization, followed by BM, BC, and WB, while GP continued to show the lowest efficiency.

Table 3.3: Feed conversion ratio, gross energy conversion ratio, and nitrogen conversion ratio of black soldier fly larvae (Hermetia illucens) fed on different subtrates 4 d, 8 d and 12 d rearing.

Item	Subtrates[1]							
	CS	GP	BM	BC	WB	WP	RSC	CON
Feed conversion ratio, in fresh matter								
D4[2]	5.2±0.4^{a}	4.1±0.3^{b}	4.2±0.3^{b}	2.5±0.1^{d}	2.4±0.1^{d}	3.1±0.2^{c}	2.7±0.3^{c}	2.5±0.1^{d}
D8[3]	4.0±0.3^{a}	3.1±0.4^{b}	2.2±0.4^{c}	2.0±0.1^{d}	1.9±0.1^{d}	2.4±0.2^{c}	2.1±0.2^{c}	1.9±0.1^{d}
D12[4]	4.2±0.4^{a}	4.0±0.3^{b}	2.1±0.1^{d}	1.9±0.1^{d}	2.0±0.0^{d}	2.4±0.1^{c}	2.8±0.3^{c}	1.9±0.3^{d}
Feed conversion ratio, in dry matter								
D4[2]	28.4±2.2^{a}	34.1±1.7^{a}	21.9±1.9^{b}	12.5±1.4^{c}	12.9±0.4^{c}	9.7±0.2^{d}	8.3±0.3^{d}	9.5±0.4^{d}
D8[3]	21.5±4.0^{a}	13.2±3.1^{b}	7.6±0.6^{c}	7.1±0.4^{c}	5.5±0.3^{cd}	7.0±0.3^{c}	6.1±0.5^{c}	5.2±0.1^{d}
D12[4]	17.8±2.6^{a}	16.1±1.8^{b}	6.8±0.6^{c}	6.3±0.2^{d}	5.9±0.4^{e}	7.1±0.2^{c}	7.6±0.6^{c}	5.5±0.1^{e}
Gross energy conversion ratio, in dry matter								
D4[2]	15.1±1.5^{b}	26.1±1.5^{a}	17.1±1.2^{b}	10.8±2.1^{c}	8.4±2.5^{d}	8.5±0.5^{d}	7.1±0.5^{e}	7.4±0.5^{de}
D8[3]	11.5±2.1^{a}	8.2±1.4^{a}	4.8±0.4^{b}	4.6±0.2^{b}	3.3±0.2^{c}	5.1±0.3^{b}	4.6±0.4^{b}	3.4±0.3^{c}
D12[4]	7.3±1.2^{ab}	8.9±1.2^{a}	3.4±0.8^{d}	3.4±0.6^{d}	3.3±0.6^{de}	4.2±0.6^{c}	5.0±1.1^{c}	3.1±0.4^{e}
Nitrogen conversion ratio, in dry matter								
D4[2]	27.8±2.7^{b}	37.9±1.8^{a}	21.9±2.0^{bc}	13.8±1.9^{d}	14.3±2.0^{d}	16.4±1.3^{c}	19.1±2.4^{c}	11.2±1.4^{e}
D8[3]	11.4±2.3^{b}	6.5±2.6^{c}	4.8±1.7^{d}	4.9±1.5^{d}	4.1±0.6^{e}	9.2±1.1^{b}	12.1±1.3^{a}	6.3±1.4^{c}
D12[4]	10.2±2.1^{b}	15.7±1.8^{a}	5.7±1.3^{c}	5.8±1.0^{c}	6.0±1.0^{c}	10.1±1.0^{a}	15.7±1.6^{a}	4.6±0.5^{d}

[1] Parameters of BSFL reared on eight different substrates categorized by nitrogen-to-gross energy ratio: carrot scraps (CS), grape pomace (GP), biscuit meal (BM), bread crumbs (BC), wheat bran (WB), wheat pulp (WP), rapeseed cake (RSC), and control diet (CON). Data are presented as least square means with standard deviations. Superscripts for substrate-specific response denote significant pairwise contrasts within time points ($^{a\text{-}e}$: D4; $^{f\text{-}j}$: D8; $^{k\text{-}o}$: D12). Experimental design: substrate replicates (n = 48), time point replicates (n = 18). Statistical analysis was performed using a general linear model (GLM) with substrate (S), time (T), and their interaction as fixed factors (S x T), and replicate as a random effect.[3]D8: Harvest at day 8 of rearing;
[4]D12: Harvest at day 12 of rearing
Feed conversion ratio = Substrate supply (kg) / larval biomass at harvest (kg)
Gross energy conversion ratio = Gross energy supply (MJ GE) /100g larval gain
Nitrogen conversion ratio = Nitrogen supply (g) / 100g larval gain

3.3 Larval body composition and retention of nitrogen and energy

Table 4 summarizes larval contents of dry matter, gross energy, protein, and chitin. All four parameters were significantly affected by substrate type, harvest time, and their interaction ($P < 0.001$).

Dry matter content increased progressively from day 4 to day 12, with clear differences among substrates. On day 4, the highest dry matter contents were observed in larvae fed WB, WP, and CON, while CS yielded the lowest values. A similar pattern was evident on day 8, with WB, RSC, and CON showing the highest values and CS remaining the lowest. By day 12, dry matter content reached its maximum across treatments, with WB, RSC, and CON maintaining the highest levels and CS and GP the lowest.

Gross energy content exhibited a distinct pattern compared to dry matter. On day 4, larvae fed BM, WP, and RSC displayed the highest gross energy values, whereas CON-fed larvae showed the lowest. At day 8, BM-fed larvae retained the highest energy content, while CS, GP, WB, and CON showed the lowest values. By day 12, BM and BC supported the highest gross energy contents, whereas CON remained the lowest.

Protein content generally increased over time, except in BM-fed larvae, which remained consistently low. Substantial differences among substrates were observed at each time point. On day 4, WB, RSC, and CON yielded the highest protein concentrations, while GP and WP showed the lowest. At day 8, WB and CON maintained the highest protein levels, whereas BM exhibited the lowest. By day 12, WB-fed larvae achieved the highest protein content, followed by CON and RSC, while BM remained the lowest.

Chitin content also increased with harvest time, with most treatments (except CS) reaching their highest values at day 12. Substrate type strongly influenced chitin concentration. On day 4, WB-fed larvae exhibited the highest chitin content, markedly exceeding all other treatments, while BM and BC showed the lowest (approximately one-quarter of WB). At day 8, CS and CON yielded the highest chitin levels, while BM remained the lowest. By day 12, CON-fed larvae displayed the highest chitin content, followed by WB and CS, whereas BM and BC continued to show the lowest values.

Table 3.4: Dry mass, gross energy, nitrogen, and chitin content of black soldier fly larvae (Hermetia illucens) fed on different subtrates for 4 d, 8 d and 12 d rearing.

Item	Subtrates^{1}							
	CS	GP	BM	BC	WB	WP	RSC	CON
Dry mass, g/kg in fresh matter								
D4^{2}	196±10^{d}	211±11^{c}	221±16^{c}	233±12^{b}	237±15^{ab}	237±12^{ab}	227±12^{b}	241±14^{a}
D8^{3}	178±29^{d}	230±11^{c}	267±21^{b}	260±14^{b}	276±27^{ab}	237±10^{c}	315±16^{a}	316±16^{a}
D12^{4}	221±17^{d}	243±22^{d}	297±27^{b}	291±28^{c}	303±32^{b}	314±18^{ab}	319±15^{ab}	321±22^{a}
Gross energy, MJ/kg in dry matter								
D4^{2}	23.1±0.9^{c}	22.9±0.9^{c}	27.3±1.8^{a}	27.0±0.3^{a}	21.9±1.2^{d}	26.9±0.9^{ab}	26.3±0.4^{b}	19.0±1.7^{d}
D8^{3}	23.1±0.9^{d}	22.9±1.2^{d}	30.3±0.5^{a}	27.8±1.5^{b}	23.5±0.5^{d}	25.2±0.9^{c}	26.1±0.4^{c}	22.7±1.4^{d}
D12^{4}	24.0±0.4^{c}	24.9±1.1^{b}	30.9±2.3^{a}	30.6±4.7^{a}	23.6±2.9^{c}	25.1±4.7^{b}	26.6±0.2^{b}	21.5±3.1^{d}
Protein^{5}, g/kg in dry matter								
D4^{2}	307±9^{b}	293±12^{c}	278±15^{d}	298±17^{b}	357±21^{a}	284±16^{c}	357±13^{a}	356±28^{ab}
D8^{3}	309±18^{c}	307±10^{c}	200±12^{d}	349±12^{b}	391±22^{a}	363±29^{b}	361±8^{b}	379±14^{ab}
D12^{4}	319±22^{d}	347±24^{c}	217±18^{e}	334±11^{c}	419±21^{a}	303±11^{d}	377±14^{b}	399±24^{b}
Chitin, g/kg in dry matter								
D4^{2}	28.2±2.0^{d}	24.8±2.1^{d}	15.4±1.4^{e}	15.8±1.9^{e}	60.3±2.6^{a}	35.1±3.7^{c}	34.7±2.9^{c}	37.3±2.9^{b}
D8^{3}	85.8±4.8^{a}	53.8±4.9^{c}	32.2±4.4^{e}	43.6±3.8^{d}	63.7±6.0^{b}	63.2±6.3^{b}	50.7±6.4^{c}	84.2±4.7^{a}
D12^{4}	78.2±4.9^{b}	62.4±5.1^{d}	50.7±3.3^{de}	45.6±4.8^{e}	80.9±5.9^{b}	68.4±6.8^{c}	81.2±5.9^{b}	91.6±5.3^{k}

[1] *Parameters of BSFL reared on eight different substrates categorized by nitrogen-to-gross energy ratio: carrot scraps (CS), grape pomace (GP), biscuit meal (BM), bread crumbs (BC), wheat bran (WB), wheat pulp (WP), rapeseed cake (RSC), and control diet (CON). Data are presented as least square means with standard deviations. Superscripts for substrate-specific response denote significant pairwise contrasts within time points ($^{a\text{-}e}$: D4; $^{f\text{-}j}$: D8; $^{k\text{-}o}$: D12). Experimental design: substrate replicates (n = 48), time point replicates (n = 18). Statistical analysis was performed using a general linear model (GLM) with substrate (S), time (T), and their interaction as fixed factors (S x T), and replicate as a random effect.*

[2] *D4: Harvest at day 4 of rearing;*

[3] *D8: Harvest at day 8 of rearing;*

[4] *D12: Harvest at day 12 of rearing*

[5] *Calculated as nitrogen × 4.76 according to Janssen et al., (2019)*

Gross energy retention, expressed as the percentage of dietary gross energy incorporated into larval biomass, was significantly affected by substrate, harvest time, and their interaction ($P < 0.001$; Table 5). Overall, energy retention increased with rearing time across all treatments. On day 4, the highest retention was observed in larvae fed BM, BC, and WP, while CON exhibited the lowest values. At days 8 and 12, BM and BC continued to show the greatest retention rates. At day 8, CS and CON displayed the lowest energy retention, whereas at day 12, CS and GP remained the least efficient substrates.

Nitrogen retention followed a similar trend, with significant effects of substrate, time, and their interaction (Table 5; $P < 0.001$). Nitrogen incorporation into larval biomass increased from day 4 to day 12 across all treatments. On day 4, WB and CON achieved the highest nitrogen retention, while CS exhibited the lowest. At days 8 and 12, WB maintained the highest retention, followed by BC and CON, whereas RSC consistently showed the lowest values.

Table 3.5: Gross energy retention and nitrogen retention of black soldier fly larvae (Hermetia illucens) fed on different subtrates for 4 d, 8 d and 12 d rearing.

Item	Subtrates^{1}							
	CS	GP	BM	BC	WB	WP	RSC	CON
Retention of gross energy, in %								
D4^{2}	8.3±1.8^{c}	9.0±1.7^{b}	11.6±2.4^{a}	10.3±3.2^{ab}	7.8±3.4^{c}	10.7±2.1^{ab}	9.8±2.5^{b}	6.5±3.5^{d}
D8^{3}	21.5±1.5^{d}	23.3±3.7^{c}	28.4±1.6^{a}	27.1±4.2^{a}	23.5±1.8^{c}	24.4±2.0^{c}	26.0±1.4^{b}	22.4±3.2^{cd}
D12^{4}	22.3±1.5^{d}	23.5±2.0^{d}	34.3±8.7^{a}	34.0±2.4^{a}	26.3±3.8^{b}	26.5±8.4^{b}	27.1±2.2^{b}	25.5±6.1^{c}
Retention of nitrogen, in %								
D4^{2}	20.6±5.5^{cd}	22.4±6.1^{c}	28.3±3.4^{b}	28.4±1.2^{b}	35.5±4.8^{a}	17.8±5.5^{d}	23.3±4.1^{c}	32.0±2.2^{a}
D8^{3}	41.1±2.3^{d}	40.8±5.1^{d}	42.3±1.1^{c}	52.2±2.4^{b}	57.7±6.1^{a}	41.0±1.5^{c}	38.6±2.3^{e}	51.1±4.8^{b}
D12^{4}	49.2±5.4^{c}	44.9±4.7^{d}	45.5±7.2^{d}	55.0±4.6^{b}	60.9±5.4^{a}	42.5±4.6^{d}	40.7±3.5^{e}	55.9±2.3^{b}

[1] *Parameters of BSFL reared on eight different substrates categorized by nitrogen-to-gross energy ratio: carrot scraps (CS), grape pomace (GP), biscuit meal (BM), bread crumbs (BC), wheat bran (WB), wheat pulp (WP), rapeseed cake (RSC), and control diet (CON). Data are presented as least square means with standard deviations. Superscripts for substrate-specific response denote significant pairwise contrasts within time points ($^{a-d}$: D4; $^{e-i}$: D8; $^{j-n}$: D12).*

Experimental design: substrate replicates (n = 48), time point replicates (n = 18). Statistical analysis was performed using a general linear model (GLM) with substrate (S), time (T), and their interaction as fixed factors (S x T), and replicate as a random effect.

[2] *D4, D8, D12 = harvest at days 4, 8, and 12 of rearing, respectively.*

Abbreviations: SEM, Standard error of means

3.4 Frass composition

Table 6 presents the composition of larval frass, including dry matter, gross energy, and nitrogen content. Significant effects of substrate and harvest time were observed for all parameters, and a significant interaction between these factors was detected for nitrogen content ($P < 0.001$).

Frass dry matter content increased from day 4 to day 12 across all treatments. The highest dry matter content was recorded on day 4 in larvae fed GP, and on days 4 and 8 in those fed GP and WP. The lowest dry matter content occurred on day 4 in BC and on days 8 and 12 in RSC.

Gross energy content of frass decreased over time in all treatments. On day 4, the highest values were found in frass from larvae fed WP or RSC, while CS and GP showed the lowest contents. At days 8 and 12, frass from RSC-fed larvae exhibited the highest gross energy content, whereas GP-fed larvae had the lowest values.

Nitrogen content of frass generally increased with time in most treatments (except RSC). Marked differences among substrates were evident. RSC consistently produced frass with the highest nitrogen content at all three time points, while CS resulted in the lowest values. GP and BM also yielded very low nitrogen contents on day 4.

Table 3.6: Dry matter, gross energy, and nitrogen content of frass of black soldier fly larvae (Hermetia illucens) fed on different subtrates for 4 d, 8 d and 12 d rearing.

Item	Subtrates[1]							
	CS	GP	BM	BC	WB	WP	RSC	CON
Dry mass, g/kg in fresh matter								
D4[2]	537±32^{b}	648±46^{a}	336±40^{e}	358±46de	440±35^{c}	532±46^{b}	396±53^{c}	381±44^{d}
D8[3]	581±46^{b}	607±42^{a}	447±37^{c}	465±44^{c}	533±38^{bc}	610±35^{a}	413±40^{d}	484±41^{c}
D12[4]	625±20^{b}	666±21^{a}	557±36^{c}	572±38^{c}	627±46^{b}	688±41^{a}	531±35^{d}	588±43^{c}
Gross energy, MJ/kg in dry matter								
D4[2]	15.0±1.7^{d}	15.1±3.8^{d}	17.4±1.5^{c}	18.1±1.9^{b}	16.5±2.4^{cd}	20.2±2.5^{ab}	20.6±2.3^{a}	18.3±4.2^{b}
D8[3]	12.9±0.9^{d}	8.3±2.1^{e}	16.0±1.3^{c}	18.8±4.2^{b}	14.3±2.6^{d}	15.5±2.5^{c}	20.5±2.5^{a}	18.1±2.7^{b}
D12[4]	10.6±3.1^{d}	7.5±2.9^{e}	14.4±2.2^{c}	16.9±3.0^{b}	12.9±3.0^{c}	14.0±3.0^{c}	18.4±4.4^{a}	17.5±4.1^{b}
Nitrogen, g/kg in dry matter								
D4[2]	12.0±1.8^{e}	13.3±2.1^{e}	12.2±2.2^{e}	16.8±2.1^{d}	19.9±3.6^{d}	39.1±3.4^{b}	55.1±4.7^{a}	28.1±3.1^{c}
D8[3]	13.1±4.5^{e}	24.9±2.5^{c}	22.2±2.3^{d}	23.0±2.7^{cd}	28.3±3.4^{b}	34.2±2.4^{b}	51.5±4.9^{a}	24.3±4.3^{c}
D12[4]	17.5±2.5^{e}	26.0±3.3^{c}	25.1±4.7^{c}	26.1±3.7^{c}	25.6±2.8^{c}	40.4±3.7^{b}	54.7±4.5^{a}	23.0±5.7^{d}

[1] *Parameters of BSFL reared on eight different substrates categorized by nitrogen-to-gross energy ratio: carrot scraps (CS), grape pomace (GP), biscuit meal (BM), bread crumbs (BC), wheat bran (WB), wheat pulp (WP), rapeseed cake (RSC), and control diet (CON). Data are presented as least square means with standard deviations. Superscripts for substrate-specific response denote significant pairwise contrasts within time points (a-e: D4; f-j: D8; k-o: D12). Experimental design: substrate replicates (n = 48), time point replicates (n = 18). Statistical analysis was performed using a general linear model (GLM) with substrate, time, and their interaction as fixed factors, and replicate as a random effect.*

[2] *D4: Harvest at day 4 of rearing;*

[3] *D8: Harvest at day 8 of rearing;*

[4] *D12: Harvest at day 12 of rearing*

4. Discussion

Influence of substrate composition on larval growth, survival rate and biomass yield in BSFL

Black soldier fly larvae are generally capable of utilizing a wide range of substrates as nutrient sources for growth. However, the efficiency of different substrates in supporting larval development varies considerably. In addition to the nutrient composition of the substrate, its physical structure (e.g., particle size) and moisture content play critical roles (Yakti et al. 2023; Froonickx et al. 2024). Our study, which included a control diet and seven distinct by-products with highly variable nutrient profiles, confirms that larval growth, survival rate, and the utilization of gross energy and nitrogen are strongly substrate-dependent. While most substrates supported larval growth comparable to the control diet, the lowest growth rates were observed for CS and GP, which contained relatively low levels of starch and crude protein but high fiber contents. Proteins (as sources of amino acids) and starch (as an energy source) are well-established as key factors for larval growth, and BSFL can efficiently digest both due to their enzymatic capabilities (Seyedalmoosavi et al., 2022). A recent study using artificial diets (wheat starch, casein, sunflower oil, cellulose) demonstrated that maximum larval final weight was achieved with diets containing 23.5% protein and 20.6% starch on a dry matter basis. Optimal protein levels were also dependent on starch content: higher starch levels allowed for lower protein requirements and vice versa. At low starch levels, additional protein is needed because more amino acids are diverted to gluconeogenesis, reducing their availability for protein synthesis (Broeckx et al., 2025). Other studies have reported optimal substrate protein contents between 15% and 22% of dry matter (Cammack and Tomberlin, 2017; Eggink et al., 2022; Oddon et al., 2022; Berggreen et al., 2025; Schneider et al., 2025a, b), with an ideal protein-to-carbohydrate ratio of approximately 1:2.8 (Berggreen et al., 2025). Given that both protein and starch levels in CS and GP were far below these optimal values, the low final larval weights observed for these substrates are likely attributable to these deficiencies. CS and GP were also rich in fiber. Although BSFL may possess some capacity for fiber degradation via intestinal microbiota, this ability appears limited and likely influenced by fiber structure (Klüber et al. 2024; Lai et al. 2024). The poor growth performance on CS and GP indicates that high fiber content cannot compensate for low starch levels. WP, despite being rich in crude protein, was also low in starch, which likely explains its poor growth outcomes. In addition to energy and amino acids, BSFL require specific lipids, such as sterols and polyunsaturated fatty acids (PUFA), which they cannot synthesize de novo (Bellezza Oddon et al. 2022, Li et al. 2022). According to a recent study, the optimal dietary fat content is relatively low (approximately 1.9% dry matter), but sunflower oil used in this study provided high levels of PUFA (linoleic acid) and phytosterols (Broeckx et al., 2025). CS and GP were also low in fat, suggesting that insufficient PUFA and sterols may have further impaired larval growth. The observation that larval growth on other substrates (BM, BC, WB, RS) was broadly comparable to the control diet, despite substantial differences in nutrient composition, supports the notion that BSFL are generally tolerant of substrate variability.

Survival rates also differed among treatments. BSFL survival is influenced by multiple factors, including nutrient composition (protein, fat, carbohydrates, minerals), substrate structure and particle size, moisture, pH, temperature, and larval density. Optimal survival has been reported at 27°C (Nayak et al., 2023; Muñoz-Delgado et al., 2025) and under conditions of adequate substrate hydration (moist but without free water) (Frooninckx et al., 2024). Higher temperatures slightly accelerate growth but reduce survival (Raimondi et al., 2020). Optimal larval density has been estimated at 2 larvae/cm² (Nayak and Klüber, 2025). Our experimental conditions adhered to these optimal parameters. Under such conditions, survival rates in most studies range from 80% to 98% (Nayak et al., 2025; Muñoz-Delgado et al., 2025; Ogello et al., 2025; Schneider, 2025a). In the present study, survival after 12 days ranged between 65% and 75% for most substrates, which is notably lower than in our previous experiments (90–98%) conducted under similar conditions (Schneider et al., 2025a,b). Those earlier studies employed diets composed of multiple feed components, suggesting that composite substrates may be more efficiently utilized by larvae, possibly due to both nutrient diversity and structural properties. Notably, CS exhibited both poor growth and low survival. Previous research indicates that starch and protein levels, as well as their ratio, strongly influence BSFL survival (Eggink et al., 2023; Cammack and Tomberlin, 2017). Thus, the low survival on CS likely reflects inadequate energy and amino acid supply, impairing protein synthesis. Reduced survival on RSC may be attributable to glucosinolate content, as diets containing camelina or crambe meal have been associated with lower BSFL survival compared to controls (Schreven et al., 2021), consistent with evidence that glucosinolates inhibit growth and development in Lepidopteran larvae (Jeschke et al., 2017).

An important metric in insect biomass production for food or feed applications is larval biomass gain, which integrates growth (fresh weight at day 12) and survival to day 12. Factors influencing these parameters have been discussed above. The lowest biomass gains were observed for CS and GP, while WB achieved the highest values, comparable to the control diet. Most other substrates yielded slightly lower biomass gains than CON and WB, whereas RSC was markedly lower due to reduced survival. As noted, these outcomes primarily reflect the low starch and crude protein contents of certain substrates.

Protein and energy retention in BSFL

An important parameter in insect larval rearing is nitrogen utilization efficiency (NUE), defined as the proportion of substrate nitrogen retained in the larvae, primarily as protein. Reported NUE values vary widely, ranging from 1% to 80% (Frooninckx *et al.*, 2023). In our study, nitrogen retention efficiency in BSFL protein at day 12 ranged between 40% and 60% across substrates. NUE is strongly influenced by protein availability and concentration, protein digestibility, amino acid composition, and the carbon-to-nitrogen (C/N) ratio of the substrate (Lu *et al.*, 2021; Frooninckx *et al.*, 2023; Schow-Madsen *et al.*, 2025). Protein digestibility is further affected by the larval gut microbiota, which produce proteolytic enzymes that facilitate protein breakdown (Yu *et al.*, 2023). In recent studies, optimal NUE were found at dietary protein concentrations of around 10% of dry matter, whereas diets with higher protein levels resulted in reduced NUE (Fuso *et al.*, 2021; Schneider *et al.*, 2025a). Excessive protein can also promote nitrogen release as ammonia and nitrous oxide (Frooninckx *et al.*, 2023; Coudron *et al.*, 2024). In our experiment, the highest NUE occurred with substrates containing 11% (BC) to 15% (WB, CON) crude protein. Substrates with lower (CS, GP, NM) or higher (WP, RSC) crude protein contents exhibited reduced NUE, consistent with other studies reporting optimal NUE within 9–15% protein (Coudron *et al.*, 2024; Schneider *et al.*, 2025a, b). The observation that substrates with very high protein levels (WP, RSC) yielded lower NUE likely reflects surplus protein that larvae cannot utilize for growth (Coudron *et al.*, 2024).

Larval protein content is a key determinant of the nutritional value of BSFL-derived meal and is primarily influenced by available protein and its amino acid profile (Schneider, 2025a; Schow-Madsen *et al.*, 2025). Energy supply, particularly starch, also plays a critical role, as protein synthesis is an energy intensive process. At low starch levels or C/N ratios below 16:1, amino acids are diverted to energy production via gluconeogenesis, reducing their availability for protein synthesis (Lu *et al.*, 2021). Under standard production conditions, BSFL protein content typically ranges from 35% to 45% (Barragan-Fonseca *et al.*, 2017; Eggink *et al.*, 2022; Kalbkol *et al.*, 2024). In our study, most substrates yielded larval crude protein contents between 30% and 42% (except BM). The highest crude protein levels were observed for WB and CON, likely due to their favorable protein and starch concentrations, which also corresponded with high NUE. Conversely, larvae fed BC exhibited markedly lower crude protein content, which we attribute to the substrate’s very high starch level, promoting lipid synthesis and reducing relative protein proportion due to increased fat deposition. Elevated energy content in these larvae supports this interpretation.

Energy and fat retention in BSFL are primarily influenced by substrate starch and fat content. Higher dietary fat increases larval fat and energy content while reducing protein levels (Li *et al.*, 2022; El-Deen *et al.*, 2023). Insects generally exhibit high capacity for triglyceride synthesis from glucose; thus, high starch or sugar levels increase fat content and energy retention (Arrese and Soulages, 2010; Spranghers *et al.*, 2016; Carpentier *et al.*, 2024). Substrates with low protein can also enhance fat retention, as less energy is allocated to protein synthesis and more to triglyceride formation (Pimentel *et al.*, 2017, Schneider *et al*., 2025a, b). Although larval fat content was not directly measured in this study, energy content of dry matter serves as an indicator of body composition, given that triglycerides (39 kJ/g) have a much higher energy density than proteins (24 kJ/g). Larvae fed BM and BC exhibited substantially higher energy content than those fed other substrates, suggesting elevated fat levels, likely due to BM and BC's high starch content. BM also contained slightly more fat than other substrates, further promoting lipid deposition. In contrast, larvae fed WB and CON had the highest crude protein content and relatively low energy content, indicating that these substrates—characterized by near-optimal protein levels (152 and 153 g/kg, respectively) and moderate starch concentrations (147 and 213 g/kg, respectively)—primarily supported protein synthesis rather than triglyceride accumulation. These substrates also achieved the most favorable feed and energy conversion ratios, reinforcing the conclusion that energy was primarily directed toward protein synthesis. Conversely, CS and GP exhibited very high feed and energy conversion ratios (indicative of poor efficiency), likely due to their low starch and crude protein contents, which limited growth and anabolic capacity.

Chitin content in BSFL

Chitin is an unbranched polysaccharide that plays a crucial role in insect larvae as the primary structural component of the cuticle. Beyond providing structural integrity and mechanical stability, chitin is essential for molting and serves as a barrier with immunological functions, protecting against pathogens (Muthukrishnan and Merzendorfer, 2016; Muthukrishnan et al., 2019; Mallick and Eleftherianos, 2024). Larval chitin content is primarily determined by developmental stage, increasing progressively throughout larval development, with the highest levels observed in prepupae and adults (Eggink and Dalsgaard, 2023; Ha et al., 2024). However, substrate composition also significantly influences chitin synthesis. Protein-rich substrates can enhance chitin content by improving nitrogen availability for chitin biosynthesis. Schneider et al. (2025a) reported that increasing substrate protein concentration from 10% to 20% of dry matter raised larval chitin content from 58 to 71 g/kg dry matter. Conversely, carbohydrate-rich substrates may reduce chitin content, as these conditions favor lipid synthesis at the expense of chitin formation (Spranghers et al., 2017). These relationships explain the chitin levels observed in our study: the highest chitin contents occurred in larvae reared on substrates with moderate to high crude protein and low to moderate starch concentrations (WB, RSC, CON), which evidently promoted chitin synthesis. In contrast, the lowest chitin contents were found in larvae fed substrates with moderate crude protein but very high starch levels (BM, BC)—the same substrates associated with the highest gross energy content in larvae. These findings suggest that under such conditions (moderate protein, high starch), lipid synthesis is favored over chitin synthesis. A relatively low chitin content was also observed for GP, likely due to its low crude protein concentration.

Chitin content in insect larvae is also relevant from a nutritional perspective. While chitin, as an indigestible carbohydrate, may exert prebiotic effects and support gut health, it can negatively affect feed digestibility in livestock (Saeb et al., 2022; Abenaim and Conti, 2025).

Composition of the frass

The residues remaining after BSFL rearing are referred to as frass, which consists of unconsumed substrate, larval excreta, and larval exuviae (Lomonaco et al., 2024). Frass is typically collected by sieving after most larvae have completed the feeding phase (Klammsteiner et al., 2020). Due to its nutrient composition, insect frass is well-suited for use as an organic fertilizer. It contains high concentrations of essential plant nutrients as well as chitin, which can stimulate plant defense mechanisms and growth (Sharp et al., 2013). The composition of frass is largely determined by the feed substrate used (Gärttling and Schulz, 2022; Lomonaco et al., 2024; Ogello et al., 2025). Our study confirms that frass composition is highly variable and closely reflects the nutrient profile of the substrate. As expected, nitrogen—a key plant nutrient—was most abundant in frass derived from substrates with the highest crude protein contents (WB, RSC), whereas substrates with low crude protein levels (CS, GP, BM) produced frass with markedly lower nitrogen concentrations. Gärttling and Schulz (2022) compiled data from 30 analyses across different studies, reporting an average nitrogen content of 32 g/kg dry matter. In our study, nitrogen concentrations in frass ranged from 17.5 to 54.7 g/kg dry matter, which aligns with this reference range. Frass with high organic nitrogen content can replace synthetic fertilizers and, due to its composition, improve soil fertility—contributing to circular bioeconomy principles. Furthermore, frass may contain beneficial microorganisms such as Lactobacillus spp., which can promote plant growth (Fuhrmann et al., 2022; Lopes, 2022; Lomonaco et al., 2024). Thus, the use of frass as a fertilizer represents an important contribution to sustainable nutrient recycling and circular bioeconomy.

5. Conclusions

This study evaluated the suitability of various food industry by-products as rearing substrates for BSFL. The results demonstrate substantial variation in larval growth, survival, biomass yield, and substrate nitrogen and energy utilization. While this investigation does not allow precise determination of individual nutrient requirements for larval growth, it clearly shows that substrates with low starch content and moderate crude protein levels (e.g., BM), combined with high fiber content (CS, GP), result in poor growth and low biomass yield. Conversely, optimal growth and biomass production were achieved with substrates containing sufficiently high protein and moderate starch concentrations (e.g., WB). High starch levels (BM, BC) were associated with reduced larval protein content and increased energy content, likely due to enhanced lipid synthesis at the expense of protein synthesis. The highest chitin concentrations—a relevant component of insect meal—were observed in larvae reared on substrates with moderate to high protein and low to moderate starch levels (WB, RSC, CON), conditions that appear to favor chitin biosynthesis. Furthermore, the study confirms previous findings that substrate composition strongly influences frass composition, particularly nitrogen content, which determines its suitability as an organic fertilizer.

Author contributions

LS and GD conceptualized the study; LS, NS, ESG, and GD developed the methodology; LS, NS, BK, SCL, and BMM conducted the investigation; LS and BK curated the data; LS drafted the manuscript; LS, CKW, RJ, KE, and GD reviewed and edited the manuscript; LS and GD managed the project; LS, ESG, and GD acquired funding. All authors contributed to writing and editing.

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4 Publication II

Dietary Protein Levels in Isoenergetic Diets Affect the Performance, Nutrient Utilization and Retention of Nitrogen and Amino Acids of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) Larvae

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Abstract

Simple Summary: Black soldier fly, *Hermetia illucens* (L.), larvae (BSFL) efficiently convert organic low-value substrates into high-value proteins, lipids, and chitin, offering solutions to global challenges in sustainable food production and biotechnological innovation. While BSFL-derived products, such as BSFL protein meal for animal feed, have been widely studied, the nutritional requirements of BSFL themselves remain insufficiently explored, and knowledge in this area is still limited. This study investigates the impact of dietary protein levels (10%, 14%, 16%, and 20% crude protein, CP) in isoenergetic diets on BSFL growth performance and nutrient utilization. Our findings indicate that larvae fed diets containing 14% protein exhibited the highest growth rates and the most efficient conversion of nutrients. In contrast, larvae on a high-protein diet (20%) accumulated the highest levels of minerals, such as calcium, yet exhibited a reduced retention of nitrogen and amino acids. On the other hand, low-protein diets (10%) led to decreased larval growth, lower chitin and higher fat deposition in the larvae. These findings highlight the importance of balancing dietary protein to optimize BSFL farming and improve sustainable production strategies.

Abstract: Black soldier fly, H. illucens larvae, efficiently convert low-value organic substrates into high-value products, offering solutions to global challenges in sustainable food production and biotechnology. This study investigated the impact of dietary protein levels (10%, 14%, 16%, and 20% crude protein, CP) on BSFL growth, nutrient utilization, and energy retention using isoenergetic diets (18.5 ± 0.3 MJ/kg dry matter) under commer-cial-scale conditions. Larvae were harvested after 8 days of feeding, with 5 replicates per treatment. Optimal growth performance and feed conversion ratios were observed in lar-vae fed 14% CP diet, with a quadratic relationship between dietary CP and biomass gain ($p < 0.001$, R2 = 0.870). Ash and calcium deposition peaked in CP20-fed larvae and were lowest in CP14-fed larvae. Phosphorus and glucosamine deposition remained unaffected, while chitin deposition correlated positively with larval weight. Nitrogen and amino acid retention were highest in CP14-fed larvae but reduced in CP20-fed larvae ($p < 0.001$, R2 = 0.573–0.902). CP10-fed larvae showed impaired growth and nitrogen deposition but in-creased fat deposition. These findings establish the CP14 diet as the optimal formulation for scalable BSFL production, providing critical insights into dietary protein effects on BSFL physiology and enabling the development of efficient feeding strategies for industri-al-scale farming.

Keywords:

black soldier fly larvae; insect nutrition; amino acids; retention

Introduction

As the global population grows and urbanization, climate change, and soil degrada-tion intensify, essential resources like water, energy, and arable land are under increasing pressure. These challenges, along with rising life expectancy and climate uncertainties, make it increasingly difficult to sustainably produce enough food to feed the world [1,2]. To meet this escalating demand, society must look beyond conventional sources and em-brace innovative, alternative resource streams that are both efficient and sustainable.

Black soldier fly H. illucens larvae represent a promising solution, capable of convert-ing low-value organic substrates—including food waste, livestock manure, and food pro-duction side streams—into high-value products such as proteins, lipids, and chitin. These bioconversion capabilities position BSFL as a sustainable resource for animal feed and bi-otechnological applications [3–10]. However, while interest in BSFL farming is growing, critical knowledge gaps remain regarding their nutritional requirements, particularly op-timal dietary protein levels, which are crucial for achieving sustainable and profitable production [11–13].

Dietary protein is essential for larval growth, biomass development, digestion, im-mune function, and hormone regulation [14,15]. Protein supplies essential amino acids (EAA) required for growth. When protein sources are deficient in EAA, larval development can be hampered [15,16]. Conversely, excessive dietary protein levels increase feed costs and contribute to environmental pollution through nitrogen excretion. Optimizing protein intake not only improves feed conversion efficiency but also reduces the environmental footprint of BSFL farming [16–19]. Existing studies highlight the importance of a balanced protein-to-energy ratio in larval diets, but the role of EAA, which are critical for growth and cannot be synthesized by larvae, remains underexplored [20–23].

For instance, Tomberlin et al. [21] identified 10 essential amino acids for BSFL, in-cluding arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threo-nine, tryptophan, and valine. These amino acids play key roles in protein synthesis and physiological functions, such as arginine’s involvement in digestion via nitric oxide pro-duction. A lack of EAA can significantly reduce larval biomass [23]. Additionally, the nu-tritional quality of BSFL products is influenced by larval feeding substrates, which affect growth rates, body composition, and survival [19,24]. For example, high-carbohydrate but low-protein diets may result in suboptimal larval growth due to EAA deficiencies.

This study aims to provide insights into optimizing BSFL nutrition by identifying the optimal protein concentration in isoenergetic diets. These findings will enhance larval growth performance and nutrient utilization, improve resource efficiency, and promote environmentally sustainable insect farming practices.

Materials and Methods

Experimental Diets

Based on the experiments conducted in our laboratory and according to previous studies [13] the study tested five different experimental diets on BSFL: four isoenergetic diets with increasing crude protein levels (10%, CP10; 14%, CP14; 16%, CP16; 20%, CP20). The standard Gainesville diet [25] was used as a control for environmental conditions (Table 1). Prior to the preparation of the experimental diets, the dry matter content of all raw materials was quantified using a precision moisture analyzer (Radwag MA 200/1.X2.IC.A, Radon, Poland). The substrates were weighed and mixed with warm tap water (27 °C) to achieve a 75% moisture content. After preparation, a 150 g representative sample was collected from each container and analyzed for nutrient concentrations (Tables 4.1 and 4.2).

Table 4.1: Ingredients (g/kg), chemical composition (g/kg), and gross energy (MJ/kg) of the experimental diets.

Items	CON	CP10	CP14	CP16	CP20
Ingredients, g/kg					
Apple pomace	-	220.0	200.0	180.0	150.0
Rape seed cake	-	5.0	140.0	140.0	239.8
Wheat pulp	-	10.0	40.0	50.0	80.0
Biscuit flour	-	220.0	275.0	225.0	155.0
Bread crumbs	-	360.0	310.0	220.0	160.0
Rape seed oil	-	10.0	10.0	10.0	8.0
Wheat bran	500.0	175.0	175.0	175.0	175.0
Alfalfa	300.0	-	-	-	-
Maize	200.0	-	-	-	-
Analysed nutrient composition [a]					
Dry matter	247	245	251	240	234
GE [MJ/kg]	16.4	18.5	18.5	18.3	18.8
Crude protein [b]	142	102	144	163	202

Items	CON	CP10	CP14	CP16	CP20
N:GE ratio [c]	1.36	0.89	1.26	1.43	1.72
Crude ash	63.0	52.6	55.6	56.9	60.3
Ether extract	24.3	72.3	70.6	68.2	62.2
aNDFom	370	237	306	313	361
Starch	187	312	260	254	220
Calcium	4.0	2.8	3.2	3.4	3.6
Phosphorus	6.8	3.5	5.5	5.7	6.9

Abbreviations: CON, control diet; CP10, 10% CP diet; CP14, 14% CP diet; CP16, 16% CP diet; CP20, 20% CP; GE, gross energy; aNDFom, amylase neutral detergent fiber organic matter. [a] *Values are means of duplicate analyses.* [b] *Calculated as nitrogen × 6.25.* [c] *Calculated as nitrogen: gross energy (N:GE).*

Table 4.2: Analyzed concentrations of amino acids of the experimental diets.

Items	CON	CP10	CP14	CP16	CP20
Total amino acids, g/kg DM	120.2	81.8	124.3	135.1	173.8
Essential amino acid composition, g/kg DM					
Arginine	7.8	4.4	7.1	7.6	10.1
Histidine	3.1	2.0	3.3	3.6	4.8
Isoleucine	5.4	3.5	5.4	5.9	7.6
Leucine	10.1	6.1	9.3	10.0	13.1
Lysine	6.9	3.6	6.0	6.1	8.8
Methionine	2.4	1.5	2.3	2.6	3.4
Phenylalanine	6.0	3.8	5.6	6.0	7.6
Threonine	5.4	3.5	5.5	6.1	8.2
Tryptophan	2.0	1.3	1.9	2.0	2.6
Valine	7.2	4.7	7.4	8.0	10.2
Non-essential amino acid composition, g/kg DM					
Alanine	8.3	5.2	8.0	8.4	10.7
Aspartic acid	11.1	7.6	10.1	11.1	13.6
Cysteine	2.2	1.7	2.9	3.2	4.2
Glutamic acid	19.8	16.2	24.5	27.3	34.3
Glycine	7.7	5.5	7.9	8.6	10.4
Proline	7.4	5.6	8.6	9.5	12.3
Serine	5.9	4.0	6.1	6.6	8.6

Abbreviations: CON, control diet; CP10, 10% CP diet; CP14, 14% CP diet; CP16, 16% CP diet; CP20, 20% CP.

Rearing Conditions

The BSF colony was obtained from a commercial breeding farm (madebymade GmbH, Pegau, Germany). Newly hatched larvae were reared on chicken feed until they were 6 days old larvae (DOL). Rearing continued in the laboratory of the Department of Animal Nutrition (University of Applied Sciences, Bingen), where larvae were sieved through 2.4 mm and 1.0 mm sieves to standardize size, removing oversized and undersized larvae. Three 1 g samples were taken and counted manually to determine the average weight of a starter larvae (7.9 ± 0.2 mg) and calculate the total mass of larvae for each container.

For the experiment, 2765 g of 6DOL were placed in a controlled climate chamber (27 ± 1.5 °C, 55 ± 5% RH) and randomly assigned to substrates. Larvae were grouped into batches of 14,000 individuals and housed in containers (40 cm × 60 cm × 12 cm; 2.1 cm3/larvae), each containing 2800 g dry mass of substrate (0.2 g DM/larvae; 0.8 g FM/larvae) and 110 g of 6DOL. The larvae were kept in containers for 8 days, during which no additional feeding or mixing was carried out. In all examined container units, fewer than 5% of the larvae reached the prepupae stage, suggesting that the larvae were nearly at the same developmental stage during the observation period. The containers were arranged in a vertically stacked system, following standard rearing practices, and were rotated once daily at the same time within the climate chamber to ensure homogene-ous environmental conditions.

Sample Collection

At harvest, larvae were separated by sieving. Test conditions were based on previous experiments to ensure proper feed consumption and dry matter collection. Larvae were manually separated, rinsed, gently dried, and weighed. Fresh matter (FM) was measured by weighing the samples immediately after collection. Substrates and larvae were stored at −18 °C. Before lyophilization, the moisture content was measured using a thermogravi-metric method (M35 Moisture Analyzer, Sartorius, Göttingen, Germany). All samples were freeze-dried within 36 h using a 20 L freeze-dryer (CHRIST, Osterode am Harz, Germany) and subsequently ground to pass through a 1 mm sieve (ZM 100, RETSCH, GmbH, Haan, Germany). For the analyses of gross energy, protein, AA, fat, neutral detergent fiber (NDF), ash as well as calcium and phosphorus 1 g of lyophilized substrate and larvae were used.

Analysis of Diet and Larval Composition

Samples were analyzed according to official standardized methods of the Association of German Agricultural Analytic and Research Institutes [26]. Gross energy (GE) was measured via bomb calorimetry (IKA C 5000, Staufen, Germany). Dry matter determination was performed according to method 3.1 [26], in which a 1 g sample was dried at 103 °C for 4 h, with timing starting once the temperature reached 103 °C. After cooling in a desicca-tor, the sample was weighed. Nitrogen concentrations of substrates and larvae were determined using a nitrogen determinator (Leco Corporation, St. Joseph, MI, USA) by the Dumas method [26,27]. AA concentrations were determined by 24-h liquidhydrolysis at 110 °C in 6 mol/L HCl followed by analysis of 16 amino acids using the Waters AccQTag Ultra chemistry on a Waters Acquity UPLC (Waters Corporation, Milford, MA, USA) as described by Wang et al. [27].

Crude protein (CP) concentration of substrates was calculated by multiplying the to-tal nitrogen content by 6.25. To account for non-protein nitrogen, the protein content of larvae was further corrected using the formula CPcor = N × 4.76, as described in [17]. Ether extracts were analyzed according to 5.11 [26] and extraction was subsequently performed using petroleum ether in a Behr E6 system (Behr Labor-Technik, Düsseldorf, Germany). Ash represents the inorganic mineral content of the sample. It was determined by pre-washing the sample with a Bunsen burner and incinerating it twice at 550 °C for 6 h in a muffle furnace, per method 8.1 [26]. The residue remaining after combustion was meas-ured to determine the crude ash content. NDF was determined by methods 6.5.1 and 6.5.2 [26], using α-amylase for NDF (Fibertherm FT 12, Gerhardt, Germany). NDF were ex-pressed without residual ash. Calcium and phosphorus were measured by ICP-OES (Quantima, GBC Scientific Equipment, Melbourne, Vic, Australia; according to method 10.8.2) [26]. Starch was analyzed by colourimetry (Roche CobasMira S, Basel, Switzerland; Randox GLUC-PAP Kit, Crumlin, UK) after enzymatic hydrolysis (Merck, 10115-5G-F, Darmstadt, Germany) according to method 7.2.1 [26].

Determination of Chitin and Glucosamine

The chitin/chitosan content in the insect samples was quantified using the method by Urs et al. [28] with a few modifications. To account for the complexity of the insect sam-ples, 5 mg freeze-dried insect samples were used instead of 1 mg. The remaining steps were carried out as previously described. The samples were suspended in 0.5 mL of 6% KOH and incubated for 90 min at 80 °C and 700 rpm to break down the crystalline struc-ture within the samples. Afterwards, the samples underwent centrifugation at 14,000× g for 10 min at 4 °C to remove the KOH. The resulting pellet was washed twice with 1 mL of phosphate-buffered saline (pH 7.4). To further break down the material into smaller parti-cles, the pellet containing the chitin and chitosan was resuspended in 250 µL of dou-ble-distilled water and homogenized using bead-beating. To fully convert all glucosamine (GlcN) units in the chitin/chitosan into N-acetylglucosamine (GlcNAc) units, the pH of the sample was first adjusted to a pH of approximately 8.5 by adding 0.5 mL of 1 M NaHCO3. Next, 50 µL of the isotopically labelled [2H6]acetic anhydride (Sigma-Aldrich, St. Louis, MO, USA) was added. The reaction mixture was incubated at 30 °C for 48 h with constant shaking at 500 rpm. After incubating the samples for 48 h at 30 °C with gentle shaking, they were freeze-dried, resuspended in 500 µL of water, and filtered using a centrifugal filter modified PES 3K (VWR International, Radnor, PA, USA). A double-isotopically la-belled internal standard R*1 (0.1 g/L) was mixed with the filtrate in a 1:1 ratio to enable the quantification of the GlcNAc units via LC-MS according to Urs et al. [28].

Calculations

To determine individual larval live weight, 100 larvae were randomly selected from each container and weighed daily (±0.001 g; KERN ADB/ADJ 200-4, Balingen, Germany) until the end of the study. The larval survival rate was determined by calculating the ratio of the final larval count to the initially estimated number of BSFL, following the method of Guillaume et al. [29]. Larval biomass gain was measured as the difference between the to-tal larval mass per container at harvest and the initial larval mass. The feed conversion ra-tio (FCR) was calculated as the ratio of distributed substrate to total larval biomass gain. Additionally, the nitrogen conversion ratio (NCR = supply of nitrogen in g per 100 g larval gain), gross energy conversion ratio (GECR = supply of GE in MJ per 100 g larval gain), and total amino acid conversion ratio (TAACR = supply of total amino acids in g per 100 g larval gain) were evaluated. The conversion parameters were determined in fresh matter (FM) and dry matter (DM). Conversion parameter in FM means distributed feed in 88% DM divided by larval gain in FM. Conversion parameters in DM were calculated as distributed feed in 88% DM divided by larval gain in DM.

To assess the efficiency of larvae in consuming and converting nutrients into bio-mass, the deposition was calculated as the product of biomass gain (g/d) multiplied with the nutrient concentration (g/kg), divided by the weight of 1000 larvae. Nutrient retention (Equation (1)) per container was calculated as the total mass of nutrients in larvae in DM divided by the total mass of nutrients in the feeding substrates in DM, according to Seyedalmoosavi et al. [30].

$$\text{Retention [\% DM]} = \text{Larval gain (g)/Substrate supply (g)} \times 100 \quad (1)$$

Statistical Analysis

The experimental unit was the rearing container (n = 5 replicate rearing containers per diet). Data were analyzed using IBM SPSS Statistics (Version 29) with ANOVA, fol-lowed by the Tukey HSD post hoc test for multiple comparisons between dietary treat-ments. The dataset included larval weight, survival rate, biomass gain, and conversion ra-tios as well as chemical analyses. All parameters were tested for normality using the Shapiro–Wilk test and for homoscedasticity using Levene’s test. When normal distribution was only achieved after a log transformation, the log-transformed data were used for sta-tistical analysis. Further, a curve fitting linear and nonlinear regression analysis was per-formed. Statistical significance was set at $p \leq 0.05$, and data are presented as means with their standard error (SE). The control diet was not included in the statistical analysis.

Results

Growth Performance and Bioconversion

At day 6, the mean larval weight was 7.9 ± 0.2 mg, with no significant differences. Significant differences in larval weight were observed starting from day 7 (Figure 4.1). Lar-vae fed with CP20 reached the highest weights between days 7 and 10. In comparison, CP16-fed BSFL showed significantly higher larval weights compared to CP-fed BSFL, alt-hough this difference was only evident until day 10. ($p < 0.001$, Figure 4.1). On day 14, the final larval weights ranged from 157.2 ± 0.2 mg for the CP10 group to 215.7 ± 0.2 mg for the CP16 group ($p < 0.001$, R2 = 0.783, Figure 4.1). The CON-fed larvae weighed 170.2 ± 0.3 mg on day 14. The survival rate of the larvae was not affected by diets (Table 4.3). The biomass gain of BSFL was influenced by dietary protein levels, with the highest biomass gains (in fresh and dry matter as well) observed in larvae fed the CP16 and CP14, compared to larvae fed the CP10 diet ($p < 0.001$, R2 = 0.870 and R2 =0.886, Table 4.3).

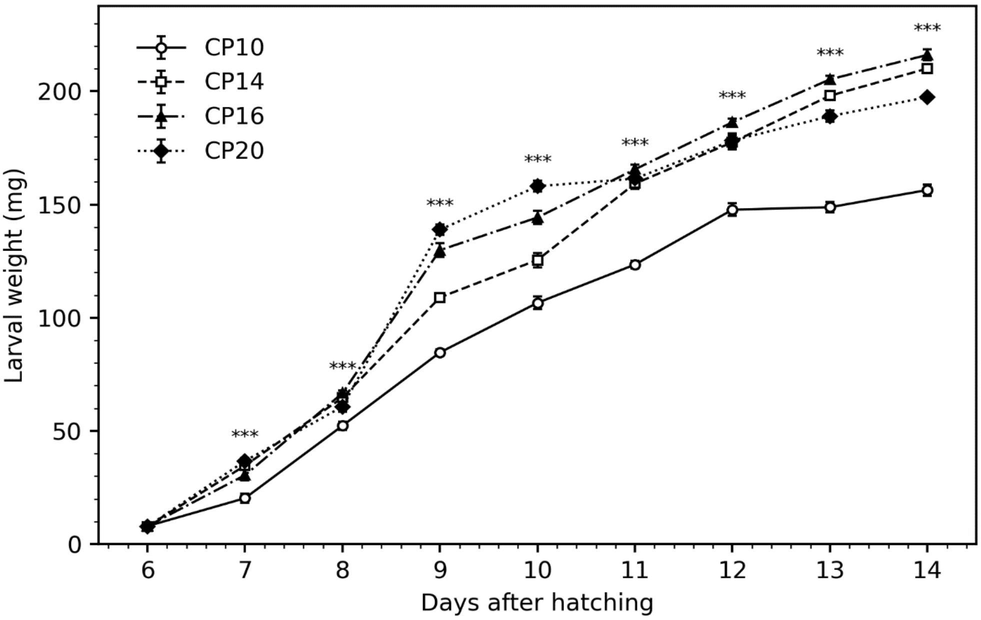

*Figure 4.1: Development of larval weight. Data are presented as means ± standard error (n = 5/diet); *** indicates the effect of dietary treatment (p < 0.001).*

Feed conversion ratio (FCR) on a fresh matter basis decreased with higher protein levels, with the lowest values recorded for CP16- and CP14-fed BSFL and the highest for CP10-fed BSFL ($p < 0.001$, R2 =0.865, Table 4.3). A similar trend was observed for FCR on a dry matter basis, where larvae reared on CP14 had the lowest value compared to larvae fed CP10 ($p < 0.001$, R2 =0.749, Table 4.3). Gross energy conversion ratio (GECR) on both fresh and dry matter bases was highest for CP10-fed BSFL compared to BSFL fed with other die-tary treatments ($p < 0.001$, R2 = 0.859 and R2 = 0.747, respectively). Nitrogen conversion ra-tio (NCR) increased linearly with higher dietary protein concentrations, with the highest value in CP20-fed BSFL and the lowest in CP10-fed BSFL on both fresh and dry matter ba-ses ($p < 0.001$, R2 = 0.921 and R2 = 0.909, Table 4.3). The total amino acid conversion (TAACR) showed a marked increase with higher dietary protein levels. On a fresh matter basis, TAACR was 65% higher in larvae fed CP20 compared to CP10. Similarly, on a dry matter basis, TAACR increased by 81% in CP20-fed larvae relative to CP10 ($p < 0.001$; R2 = 0.875 and R2 = 0.893, Table 4.3).

Table 4.3: Growth performance and conversion parameters of Hermetia illucens larvae.

Parameter	Dietary Treatments					SE	*p*-Value
	CON	CP10	CP14	CP16	CP20		
Survival rate, %	97.5	90.4	94.6	93.8	92.7	0.04	0.677
Biomass gain [1], kg FM	1.63	1.51 ^{c}	2.07 ^{a}	2.15 ^{a}	1.93 ^{b}	5.40	<0.001
Biomass gain [1], kg DM	0.53	0.50 ^{c}	0.65 ^{a}	0.64 ^{a}	0.58 ^{b}	1.41	<0.001
FCR [2], FM	2.0	1.7 ^{a}	1.2 ^{c}	1.2 ^{c}	1.4 ^{b}	0.04	<0.001
FCR [2], DM	5.9	5.4 ^{a}	4.2 ^{c}	4.3 ^{c}	4.7 ^{b}	0.11	<0.001
GECR [3], FM	2.9	3.6 ^{a}	2.6 ^{b}	2.5 ^{b}	2.9 ^{b}	0.09	<0.001
GECR [3], DM	9.1	11.0 ^{a}	8.4 ^{b}	8.5 ^{b}	9.3 ^{b}	0.24	<0.001
NCR [4], FM	4.8	3.7 ^{c}	3.9 ^{b}	4.2 ^{b}	5.7 ^{a}	0.15	<0.001
NCR [4], DM	14.8	11.3 ^{d}	12.6 ^{cd}	14.1 ^{bc}	18.9 ^{a}	0.56	<0.001
TAACR [5], FM	21.4	15.8 ^{c}	17.4 ^{b}	18.3 ^{b}	26.1 ^{a}	0.77	<0.001
TAACR [5], DM	66.1	48.0 ^{c}	55.5 ^{b}	61.7 ^{b}	86.8 ^{a}	2.79	<0.001

a–c: Values in a row that are marked without the same superscript letter differ significantly ($p < 0.05$). Data are presented as means (n = 5/diet), standard error (SE), and p-value. Abbreviations: CON, control diet; CP10, 10% CP diet; CP14, 14% CP diet; CP16, 16% CP diet; CP20, 20% CP; FM, fresh matter; DM, dry matter. The control diet was not included in the statistical analysis. [1] Calculated as harvest weight per container (kg)—initial weight per container (kg). [2] FCR = Feed supply (g)/larval gain (g). [3] GECR = gross energy supply (MJ GE)/100 g larval gain. [4] NCR = nitrogen supply (g)/100 g larval gain. [5] TAACR = total amino acid supply (g)/100 g larval gain.

Nutrient, Mineral and Amino Acid Composition

Table 4.4 presents the analyzed nutrient composition of BSFL dry biomass. Larval wa-ter concentration showed no significant differences among treatments. Gross energy con-centration similarly exhibited no significant differences. Larval protein concentration was strongly affected by dietary protein levels. The lowest protein concentration was observed in the larvae fed the CP10 diet, compared to all other diets (p < 0.001; R2 = 0.828, Table 4.4). Fat concentration in larval dry biomass also displayed a significant quadratic response to dietary protein levels. The highest fat concentration was recorded in larvae fed the CP10 diet, while the lowest was analyzed in the CP14-fed group (p < 0.001; R2 = 0.693, Table 4.4). In contrast, ash concentration increased linearly with higher dietary protein levels, with the highest ash concentration found in larvae fed the CP20 diet compared to CP10 group (p < 0.001; R2 = 0.968, Table 4.4). Calcium concentration was significantly higher in larvae fed the CP20 and CP16 diets compared to those larvae fed diets with lower protein con-tents (p = 0.003; R2 = 0.575, Table 4.4). Phosphorus concentration, however, showed no consistent trends across treatments. The calcium-to-phosphorus (Ca/P) ratio was highest in CP20-fed larvae and lowest in CP14-fed larvae (p = 0.014; R2 = 0.475, Table 4.4). Larval chitin concentration exhibited significant variation across the dietary treatments, with the lowest concentration observed in larvae fed the CP10 diet, in comparison to larvae fed diets with higher protein levels (p < 0.001; R2 = 0.718, Table 4.4). No significant effect of dietary protein levels was observed on glucosamine concentration in BSFL dry biomass (p = 0.799; R2 = 0.059, Table 4.4).

Table 4.4: Concentration of water, gross energy, nutrients, and minerals in biomass of Hermetia illucens larvae.

Item	Dietary Treatments					SE	*p*-Value
	CON	CP10	CP14	CP16	CP20		
Water, g/kg FM	684	680	693	708	704	2.89	0.082
GE, MJ/kg DM	23.6	27.0	26.7	27.2	27.7	1.13	0.183
Nutrients, g/kg DM							
Protein [1]	377	305	365 ^{a}	354 ^{a}	348 ^{ab}	7.05	<0.001
Fat	250	311 ^{a}	271 ^{c}	277 ^{bc}	289 ^{b}	4.59	<0.001
Chitin	63.6	58.2 ^{b}	69.4 ^{a}	67.7 ^{a}	70.8 ^{a}	2.89	<0.001
Glucosamine	1.5	1.5	1.6	1.3	1.7	0.11	0.799
Ash	102	67 ^{c}	71 ^{b}	74 ^{b}	91 ^{a}	2.84	<0.001
Minerals, g/kg DM							
Calcium	20.9	11.7 ^{b}	11.9 ^{b}	13.9 ^{a}	15.4 ^{a}	7.60	0.003
Phosphorus	9.2	6.2	6.6	6.3	6.8	2.52	0.135
Ca/P ratio, g/g DM	2.3	1.9 ^{ab}	1.8 ^{b}	2.2 ^{ab}	2.3 ^{a}	0.76	0.014

$^{a–c}$: Values in a row that are marked without the same superscript letter differ significantly (p < 0.05). Data are presented as means (n = 5/diet), standard error (SE), and p-value. Abbreviations: CON, control diet; CP10, 10% CP diet; CP14, 14% CP diet; CP16, 16% CP diet; CP20, 20% CP; GE, gross energy. The control diet was not included in the statistical analysis. [1] Calculated as nitrogen × 4.76 (according to [17]).

The sum of larval AA was affected in a quadratic manner by dietary protein levels, with the highest value observed in the CP14-fed larvae and the lowest in the CP10 group (p = 0.001; R2 = 0.796, Table 4.5). EAA, including arginine, histidine, isoleucine, leucine, ly-sine, methionine, phenylalanine, threonine, tryptophan, and valine, showed significant variation across dietary treatments. The CP14 diet generally resulted in the highest con-centrations of these AA. Non-essential AA (NEAA) such as aspartic acid, glutamic acid, glycine, proline, and serine also varied significantly, with CP14 consistently showing the highest concentrations for aspartic acid, glutamic acid, and glycine ($p < 0.001$; R2 = 0.751 to 0.625, Table 4.5). Cysteine concentration in larvae dry biomass did not show any significant difference across treatments.

Table 4.5: Concentration of amino acids in biomass of Hermetia illucens larvae.

Item	Dietary Treatments					SE	*p*-Value
	CON	CP10	CP14	CP16	CP20		
Total amino acids, g/kg DM							
Sum of AA	304.06	248.94 ^{b}	307.46 ^{a}	291.37 ^{a}	282.03 ^{a}	4.30	0.001
Essential amino acids, g/kg DM							
Sum of EAA	152.66	124.94 ^{b}	157.03 ^{a}	148.04 ^{a}	142.25 ^{a}	2.04	<0.001
Arginine	14.93	12.86 ^{b}	16.68 ^{a}	15.26 ^{a}	14.58 ^{a}	0.48	0.033
Histidine	9.41	7.51 ^{b}	10.46 ^{a}	10.16 ^{a}	9.35 ^{a}	0.27	0.001
Isoleucine	15.20	13.00 ^{b}	15.65 ^{a}	14.58 ^{a}	14.19 ^{ab}	0.22	<0.001
Leucine	27.45	23.65 ^{c}	28.29 ^{a}	26.67 ^{ab}	25.81 ^{bc}	0.40	0.001
Lysine	21.99	16.48 ^{b}	21.10 ^{a}	20.02 ^{a}	19.43 ^{a}	0.44	<0.001
Methionine	6.13	5.02 ^{c}	6.36 ^{a}	5.92 ^{ab}	5.73 ^{b}	0.10	<0.001
Phenylalanine	14.47	11.24 ^{b}	14.25 ^{a}	13.39 ^{a}	12.85 ^{ab}	0.28	0.001
Threonine	13.51	11.13 ^{c}	13.78 ^{a}	13.03 ^{ab}	12.53 ^{b}	0.21	<0.001
Tryptophan	7.48	5.88 ^{b}	7.76 ^{a}	7.70 ^{a}	7.14 ^{a}	0.19	<0.001
Valine	22.09	18.17 ^{c}	22.70 ^{a}	21.31 ^{ab}	20.64 ^{b}	0.37	<0.001
Non-essential amino acids, g/kg DM							
Sum of NEAA	151.40	124.00 ^{b}	150.43 ^{a}	143.33 ^{a}	139.78 ^{a}	1.82	<0.001
Alanine	25.5	19.87	23.86	22.91	22.28	0.62	0.222
Aspartic acid	30.6	24.09 ^{b}	31.93 ^{a}	30.04 ^{a}	28.93 ^{a}	0.65	<0.001
Cysteine	2.7	2.02	2.31	2.25	2.38	0.06	0.065
Glutamic acid	36.6	30.13 ^{b}	35.72 ^{a}	33.78 ^{ab}	33.35 ^{ab}	0.51	0.001
Glycine	20.6	16.56 ^{b}	20.69 ^{a}	19.81 ^{a}	19.21 ^{a}	0.39	0.001
Proline	21.6	19.24 ^{b}	21.50 ^{a}	20.85 ^{a}	20.55 ^{ab}	0.26	0.002
Serine	13.8	12.09 ^{b}	14.42 ^{a}	13.69 ^{ab}	13.08 ^{ab}	0.23	0.005

$^{a–c}$: Values in a row that are marked without the same superscript letter differ significantly ($p < 0.05$); Data are presented as means (n = 5/diet), standard error (SE), and p-value. Abbreviations: CON, control diet; CP10, 10% CP diet; CP14, 14% CP diet; CP16, 16% CP diet; CP20, 20% CP. The control diet was not included in the statistical analysis.

Deposition of Nutrients, Minerals and Amino Acids

Water deposition was quadratically affected by dietary protein concentration, with the highest values observed in larvae fed the CP16 diet, while the larvae fed the CP 10 diet had the lowest deposition (p = 0.022; R2 = 0.601, Table 4.6). In contrast, gross energy deposi-tion showed no significant variation across the treatments. Protein deposition was affected by the dietary treatments, with the highest deposition observed in larvae fed the CP14 diet, followed by those on the CP16 diet, compared to larvae fed the CP10 diet (p = 0.001; R2 = 0.851, Table 4.6). Fat deposition was highest in CP10-fed larvae, compared to larvae fed the CP14 and CP16 diets (p = 0.002; R2 = 0.624, Table 4.6). Chitin deposition was lowest in the CP10-fed larvae compared to those fed higher protein diets (p = 0.002; R2 = 0.594, Table 4.6). Ash deposition increased linearly with higher dietary protein concentrations, with the lowest deposition of ash in larvae fed with CP10 diet (p < 0.001; R2 = 0.961, Table 4.6). Cal-cium deposition was 35% higher in larvae fed the CP16 and CP20 diets compared to those fed the CP10 diet (p = 0.003; R2 = 0.575, Table 4.6). While larval glucosamine and phospho-rus deposition showed no significant differences between the dietary treatments.

Table 4.6: Deposition of water, energy, nutrients, and minerals per 1000 larvae of Hermetia illucens larvae.

Item	Dietary Treatments					SE	*p*-Value
	CON	CP10	CP14	CP16	CP20		
Water, g FM/d	10.3	10.1 ^{b}	13.5 ^{ab}	14.5 ^{a}	13.1 ^{ab}	0.39	0.022
GE, MJ kg DM/d	0.12	0.15	0.16	0.16	0.16	0.05	0.122
Nutrients, g DM/d							
Protein [1]	1.85	1.50 ^{b}	2.25 ^{a}	2.16 ^{a}	1.97 ^{ab}	0.08	0.001
Fat	1.26	1.78 ^{a}	1.60 ^{b}	1.62 ^{b}	1.69 ^{ab}	0.04	0.002
Chitin	0.30	0.27 ^{b}	0.35 ^{a}	0.35 ^{a}	0.42 ^{a}	0.02	0.002
Ash	0.50	0.32 ^{b}	0.44 ^{a}	0.45 ^{a}	0.51 ^{a}	0.01	<0.001
Glucosamine mg DM/d	7.3	7.6	8.5	8.2	8.7	0.65	0.799
Minerals, mg DM/d							
Calcium	100.9	57.9 ^{c}	72.9 ^{b}	84.3 ^{a}	86.9 ^{a}	3.42	0.004
Phosphorus	44.9	30.6	40.7	38.2	38.3	1.32	0.135

$^{a–c:}$ *Values in a row that are marked without the same superscript letter differ significantly (p < 0.05). Data are presented as means (n = 5/diet), standard error (SE), and p-value. Abbreviations: CON, control diet; CP10, 10% CP diet; CP14, 14% CP diet; CP16, 16% CP diet; CP20, 20% CP. The control diet was not included in the statistical analysis.* [1] *Calculated as nitrogen × 4.76 according to [17]. Deposition = Biomass gain (g/d) x nutrient (g/kg)/1000 larvae.*

The deposition of EAA in BSFL was significantly influenced by dietary protein con-tent. The lowest AA deposition was observed in larvae fed the CP10 diet, while higher deposition occurred in larvae fed diets with higher protein levels (p = 0.001; R2 = 0.815, Table 4.7). Most EAA, such as arginine, histidine, leucine, and valine, were deposited in the highest amounts in larvae fed the CP14 and CP16 diets, with the CP10 group showing the lowest values (p < 0.05, Table 4.7). Total AA deposition followed a quadratic pattern, with CP14 generally yielding the highest values (p < 0.001; R2 = 0.606 to 0.835, Table 4.7).

Table 4.7: Deposition of amino acids per 1000 larvae of Hermetia illucens larvae.

Item	Dietary Treatments					SE	*p*-Value
	CON	CP10	CP14	CP16	CP20		
Total amino acids, g DM/d							
Sum of AA	1.45	1.19 ^{b}	1.84 ^{a}	1.74 ^{a}	1.65 ^{a}	0.05	0.001
Essential amino acids, mg DM/d							
Arginine	73.1	63.0 ^{b}	102.5 ^{a}	92.7 ^{a}	87.1 ^{a}	4.02	0.002
Histidine	46.1	36.8 ^{b}	64.3 ^{a}	61.5 ^{a}	52.7 ^{ab}	2.45	<0.001
Isoleucine	74.1	63.8 ^{b}	96.0 ^{a}	88.2 ^{a}	79.9 ^{ab}	2.63	<0.001
Leucine	134.0	116.0 ^{b}	173.6 ^{a}	161.4 ^{a}	145.4 ^{ab}	4.90	<0.001
Lysine	107.1	80.8 ^{b}	129.4 ^{a}	121.1 ^{a}	109.5 ^{ab}	3.88	<0.001
Methionine	29.9	24.6 ^{b}	39.0 ^{a}	35.8 ^{a}	32.3 ^{a}	1.14	<0.001
Phenylalanine	70.4	55.1 ^{b}	87.3 ^{a}	81.0 ^{a}	72.4 ^{ab}	2.55	<0.001
Threonine	65.9	54.6 ^{b}	84.5 ^{a}	78.9 ^{a}	70.6 ^{ab}	2.41	<0.001
Tryptophan	36.3	28.9 ^{b}	47.6 ^{a}	46.7 ^{a}	40.1 ^{a}	1.70	<0.001
Valine	107.7	89.2 ^{b}	139.2 ^{a}	129.0 ^{a}	116.2 ^{a}	4.01	<0.001
Non-essential amino acids, mg DM/d							
Alanine	123.8	97.7 ^{b}	146.0 ^{a}	138.2 ^{a}	125.3 ^{a}	4.43	0.004
Aspartic acid	149.4	118.0 ^{b}	196.0 ^{a}	181.8 ^{a}	163.0 ^{ab}	6.38	<0.001
Cysteine	13.2	9.9 ^{b}	14.1 ^{a}	13.6 ^{a}	13.4 ^{a}	0.38	<0.001
Glutamic acid	178.7	147.8 ^{b}	210.0 ^{a}	204.3 ^{a}	187.7 ^{a}	5.72	<0.001
Glycine	100.6	81.2 ^{b}	127.0 ^{a}	120.0 ^{a}	108.2 ^{ab}	3.93	<0.001
Proline	105.6	94.3 ^{b}	131.9 ^{a}	126.1 ^{a}	115.5 ^{a}	3.46	<0.001
Serine	67.3	59.3 ^{b}	88.5 ^{a}	83.0 ^{a}	73.7 ^{a}	2.64	<0.001

$^{a–b}$: Values in a row that are marked without the same superscript letter differ significantly (p < 0.05). Data are presented as means (n = 5/diet), standard error (SE), and p-value. Abbreviations: CON, control diet; CP10, 10% CP diet; CP14, 14% CP diet; CP16, 16% CP diet; CP20, 20% CP. The control diet was not included in the statistical analysis.

Retention Efficiency of Gross Energy, Nitrogen and Amino Acids

Gross energy retention was higher in larvae fed the CP14 and CP16 diets compared to those on the CP10 diet (p = 0.001; R2 = 0.705, Table 4.8). Nitrogen retention also varied, with the highest levels observed in larvae fed the CP14 and CP10 diets, and the lowest in CP20-fed larvae (p < 0.001; R2 = 0.845, Table 4.8). Similarly, total AA retention was highest in larvae fed the CP14 and CP10 diets and lowest in larvae fed the CP20 diet (p < 0.001; R2 = 0.881, Table 4.8). Retention of EAA, including arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, was higher in larvae fed the CP14 diet compared to larvae fed the CP20 diet (p < 0.001; R2 = 0.625 to 0.902, Table 4.8). For NEAA, aspartic acid, glutamic acid, and glycine showed higher retention in larvae fed the CP14 diet, while cysteine and proline retention were also significantly higher in the CP10 and CP14 diets compared to the CP20 group (p < 0.05, Table 4.8).

Table 4.8: Retention (in %) of gross energy, nitrogen, and amino acids of Hermetia illucens larvae.

Item	Dietary Treatments					SE	*p*-Value
	CON	CP10	CP14	CP16	CP20		
Gross energy	27.1	25.0 ^{b}	32.2 ^{a}	32.6 ^{a}	29.7 ^{a}	0.74	0.001
Nitrogen	53.8	56.9 ^{ab}	61.6 ^{a}	53.3 ^{b}	39.1 ^{c}	1.69	<0.001
Total amino acids	48.3	54.5 ^{ab}	58.0 ^{a}	49.3 ^{b}	33.9 ^{c}	1.82	<0.001
Essential amino acids, mg/d							
Arginine	36.0	51.3 ^{a}	54.6 ^{a}	45.6 ^{a}	30.1 ^{b}	2.33	<0.001
Histidine	55.6	66.2 ^{a}	72.9 ^{a}	64.1 ^{a}	40.6 ^{b}	2.58	<0.001
Isoleucine	52.9	65.8 ^{a}	67.9 ^{a}	56.3 ^{b}	39.2 ^{c}	2.27	<0.001
Leucine	51.5	68.6 ^{ab}	71.0 ^{a}	60.3 ^{b}	41.1 ^{c}	2.41	<0.001
Lysine	67.8	80.1 ^{a}	82.1 ^{a}	75.2 ^{a}	46.1 ^{b}	2.88	<0.001
Methionine	49.0	60.5 ^{ab}	63.3 ^{a}	52.7 ^{b}	35.2 ^{c}	2.16	<0.001
Phenylalanine	45.2	52.1 ^{a}	58.8 ^{a}	50.4 ^{a}	35.6 ^{b}	1.77	<0.001
Threonine	47.5	56.6 ^{a}	57.8 ^{a}	48.2 ^{b}	32.0 ^{c}	2.00	<0.001
Tryptophan	71.1	80.1 ^{a}	92.8 ^{a}	87.8 ^{a}	57.6 ^{b}	3.10	<0.001
Valine	58.0	68.4 ^{ab}	71.6 ^{a}	60.5 ^{b}	42.1 ^{c}	2.29	<0.001
Non-essential amino acids, mg/d							
Alanine	57.6	68.1 ^{a}	69.5 ^{a}	61.8 ^{a}	43.5 ^{b}	2.53	0.003
Aspartic acid	52.2	56.0 ^{b}	73.3 ^{a}	61.5 ^{b}	44.3 ^{c}	2.20	<0.001

Item	Dietary Treatments					SE	p-Value
	CON	CP10	CP14	CP16	CP20		
Cysteine	23.7	21.2 ^{a}	18.6 ^{ab}	16.1 ^{b}	11.7 ^{c}	0.97	<0.001
Glutamic acid	34.9	33.0 ^{a}	33.9 ^{a}	28.2 ^{b}	20.3 ^{c}	2.20	<0.001
Glycine	50.3	53.6 ^{a}	60.8 ^{a}	52.4 ^{a}	38.5 ^{b}	1.70	<0.001
Proline	55.2	60.6 ^{a}	57.9 ^{a}	49.7 ^{b}	34.9 ^{c}	2.04	<0.001
Serine	44.2	43.0 ^{b}	57.8 ^{a}	47.0 ^{b}	31.6 ^{c}	1.84	<0.001

$^{a–c}$: Values in a row that are marked without the same superscript letter differ significantly ($p < 0.05$). Data are presented as means ($n = 5$/diet), standard error (SE), and p-value. Abbreviations: CON, control diet; CP10, 10% CP diet; CP14, 14% CP diet; CP16, 16% CP diet; CP20, 20% CP. The control diet was not included in the statistical analysis.

Discussion

To our knowledge, no prior research has explored the effect of isoenergetic diets with varying protein concentrations on nutrient, mineral, and AA deposition and retention efficiency in Hermetia illucens larvae. This study provides novel insights in these nutritional aspects, identifying optimal dietary protein concentrations under isoenergetic conditions for rearing BSFL.

Growth Performance

In this study, dietary protein concentrations of 14% and 16% (CP14, CP16), with a gross energy content of 18.5 ± 0.3 MJ/kg dry matter, were identified as optimal for maximizing larval growth and biomass gain. These findings underscore the critical role of dietary protein in BSFL growth performance, aligning with previous research [11,12,13,31]. Oddon et al. [13] reported that dietary protein levels between 14% and 16% optimize larval development, aligning with our findings. In our study, a quadratic relationship between dietary protein content and larval performance was observed, suggesting that while adequate dietary protein is essential, dietary protein concentrations of 10% lead to decreased growth performance and biomass gain, as the AA supply becomes insufficient to support crucial metabolic and developmental functions. Nguyen et al. [20] found that dietary protein levels above a certain threshold do not increase larval biomass gain proportionally but reduce feed intake of larvae. This reduction in feed intake is likely a physiological response to the increased metabolic costs associated with processing excess dietary protein and a longer satiety. In larvae fed the 20% CP diet, a potential decreased feed intake may explain the lower body weights observed, as reduced intake limits the available nutrients for growth while the prolonged satiety further reduces overall nutrient consumption. Furthermore, BSFL reared on high-protein diets (32–50% crude protein, dry matter basis) showed elevated mortality rates [29,32]. Excess protein catabolism and excretion, resulting from diets with high protein levels, can lead to toxic metabolites, organ damage, and increased mitochondrial reactive oxygen species [29,31,32,33,34]. In Dipteran species, excess uric acid is converted to ammonium, contributing to harmful ammonia emissions [35]. The insulin-like growth factor (IGF) and TOR signaling pathways control body size, growth, and lifespan in insects, with nutrition being a key factor in these processes. In addition to IGF and TOR, the endocrine system, particularly through the hormones ecdysteroids and juvenile hormone, plays a vital role in regulating insect growth [20,21,36,37,38,39]. Proper dietary protein contents are essential for efficient larval growth and development, as excessive protein can interfere with these metabolic processes [39]. This suggests that a diet with 14% protein content and 18.5 MJ/kg dry matter may provide an optimal balance, fulfilling the larvae's nutritional requirements while avoiding inefficiencies from excessive protein intake. The results of this study support the importance of balancing protein intake to promote larval growth performance and biomass production while reducing nitrogen loss as indicated by previous studies [12,13,14,15,40,41,42,43,44,45].

Bioconversion Efficiency

Larvae fed CP16 and CP14 diets exhibited the lowest feed conversion ratios (FCR) for both fresh and dry matter, indicating optimal feed-to-biomass conversion compared to other protein levels. This highlights the importance of balanced dietary protein in insect nutrition for efficient nutrient conversion and biomass gain [31,46,47]. Such efficiency enhances the economic viability of BSFL farming by reducing feed costs and increasing yields. However, protein requirements may vary with protein source quality, as shown in BSFL and other insects [18,48,49,50]. The gross energy conversion ratio (GECR) and nitrogen conversion ratio (NCR) also highlight the metabolic efficiency of larvae fed with optimal protein concentrations in their diet (14% and 16%). Seyedalmoosavi et al. [30] found a GECR ranging from 3.1 to 3.2 MJ/kg/100 g BSFL gain on a fresh matter basis, which is in line with the values observed in this study, ranging from 2.6 to 3.6. The observed differences in GECR values could also be influenced by variations in feed composition and the specific nutrient ratios in the diets used across studies. The lower NCR values observed in this study compared to those reported by Seyedalmoosavi et al. [30] could be attributed to differences in protein quality. Variations in the AA composition and bioavailability of the AA sources used in the diets may have led to less efficient nitrogen utilization in the study of Seyedalmoosavi et al. [30], whereas the protein sources used in this study may have supported more efficient nitrogen retention and metabolism. High NCR values in CP20-fed larvae suggest excessive protein intake leads to higher nitrogen excretion, consistent with findings by Pang et al. [47] and Gebremikael et al. [49], which note environmental concerns related to nitrogen pollution in insect farming. Excess nitrogen in the form of ammonia can lead to eutrophication and increased greenhouse gas emissions [47,48]. Variations in conversion parameters indicate that BSFL adapt their metabolism to dietary protein intake [50,51,52,53]. Insufficient protein intake (CP10) leads to impaired growth and potential nutritional stress. Conversely, excessive protein (CP20) results in diminishing returns, likely due to metabolic pathway saturation, wherein the capacity of the larvae metabolic systems to efficiently process and utilize the excess protein is surpassed, leading to inefficiencies in nutrient assimilation and increased excretion of nitrogen [23,45].

Body Composition, Deposition and Retention

The diet significantly influenced the body composition of BSFL, particularly affecting the larval protein and fat content. In terms of nutrient deposition and retention efficiency, the study found that BSFL utilize and store available nutrients differently based on the diet composition. CP14 was identified as the most optimal protein level because it resulted in the highest protein deposition while maintaining efficient feed conversion and nutrient retention. Although larvae fed CP16 exhibited similar performance in some parameters, increasing dietary protein beyond CP14 did not lead to proportional improvements in growth or nutrient utilization. Conversely, a diet with lower protein content (CP10), along with higher digestible carbohydrate contents, led to a decrease in protein synthesis and an increase in fat deposition [22,54,55].

When AA are sufficiently available, the larvae prioritize protein synthesis over fat storage, resulting in a reduction of fat content [16,22]. This is particularly significant in the production of high-quality BSFL protein meals for livestock nutrition, where the goal is to maximize protein content while minimizing the presence of ash and fat [5,6,7,8,9,10]. Additionally, this study found that the CP20 diet did not further increase protein levels in the larvae, suggesting diminishing returns from higher dietary protein concentrations, likely due to a lack of additional dietary energy sources.

The energy content of a diet is a critical determinant of larval metabolic efficiency and nutrient partitioning. Optimal dietary energy availability facilitates the efficient utilization of dietary protein, ensuring that amino acids are directed toward anabolic processes rather than catabolized for energy [36,56]. When the protein-to-energy ratio in diet is balanced, larvae prioritize protein synthesis for growth, enzymatic activity, and structural protein deposition, including cuticle components such as chitin, which are modulated by ecdysteroids [36]. Excess dietary energy relative to dietary protein intake shifts metabolism towards lipogenesis, resulting in increased larval fat deposition rather than enhanced larval protein retention [56]. Conversely, a dietary energy deficit can impair protein utilization, as amino acids are increasingly oxidized to meet energetic demands rather than being incorporated into larval biomass. In this study, diets were formulated with an energy concentration of 18.5 ± 0.3 MJ/kg dry matter, a level previously identified as optimal in BSFL nutrition. This dietary energy level ensures that dietary protein is efficiently utilized for growth rather than being diverted for maintenance metabolism, thereby supporting optimal larval development and biomass composition.

Chitin concentration was lowest in larvae fed the CP10 diet, suggesting that insufficient dietary protein limits chitin synthesis, a key structural component of the exoskeleton [50]. As chitin is composed of nitrogen-containing polysaccharides and derives partially from amino acid metabolism, a low-protein diet may restrict its biosynthesis due to limited dietary nitrogen and amino acids availability. Despite increased chitin concentration of larvae fed with higher-protein diets, glucosamine levels, a chitin biosynthesis derivative, showed no significant differences, indicating a consistent demand for structural integrity, influenced by BSFL development stage. This correlation between body weight and chitin deposition is not unique to BSFL, as analogous relationships exist in other livestock species, where skeletal development and structural protein accumulation, such as collagen and keratin formation, contribute to overall growth and body composition. However, the skeletal mass in pigs and poultry increases at a lower rate than muscle mass, resulting in a decreasing relative skeletal proportion as body weight increases. This is also consistent with the observations in this study, which found that chitin deposition increases with body mass, which may reflect its role in structural integrity and growth adaptation. Similarly, in crustaceans and fish with exoskeletal components, chitin accumulation increases with body size, emphasizing its role in structural reinforcement and growth adaptation [57]. Furthermore, the linear increase in ash and calcium concentrations in CP16- and CP20-fed larvae likely reflects a carry-over effect from the higher mineral content in protein-rich diets [19,30]. Additionally, residual dietary minerals in the digestive tract and increased mineral requirements for exoskeletal development may contribute to the elevated ash content. Thus, balancing dietary protein and dietary mineral content is essential for optimizing larval growth, nutrient retention efficiency, and biomass quality of BSFL.

Essential Amino Acid (EAA) Dynamics

When considering protein requirements, the quality and biological value of protein sources are crucial. Despite their importance, EAA are often overlooked in insect nutrition research, although they play a key role in optimizing waste digestion in BSFL production due to their varying nutrient profiles. Since animal cells cannot synthesize the carbon skeletons of EAA, these AA are crucial, as mentioned before, for synthesizing proteins re-quired for survival, growth, development, and reproduction, including enzymes, structur-al proteins, transport proteins, immune proteins, and hormones [21,23,36–39]. The current study supports this by showing that feeds with limited AA content, such as CP10, result in reduced protein and AA deposition in larvae. EAA are essential for protein synthesis and other physiological functions, as noted by Tomberlin et al. [21], who highlighted the role of EAA in promoting efficient protein synthesis in BSFL. This relationship reflects the fine balance required to provide sufficient EAA for processes such as protein synthesis, exo-skeleton, and enzymatic activity, without exceeding levels that could lead to inefficiencies or increased excretion. For example, methionine is essential for the synthesis of cysteine, which plays a critical role in the formation of structural proteins such as collagen and keratin in skin, hair, and feathers [58], and may also be involved in chitin synthesis. Simi-larly, the increased levels of leucine and lysine in the CP14 group compared to the CP10 diet indicate that this diet contains the building blocks for protein synthesis, which are required for larval growth and metamorphosis. While the larvae fed a CP14 diet had the highest total AA content in biomass, it is important to note that too much of one AA rela-tive to others can cause an imbalance that affects growth and metabolism [23]. For exam-ple, an excess of lysine in the absence of sufficient methionine and threonine can reduce growth efficiency as the excess lysine cannot be fully utilized for protein synthesis [14–16]. Furthermore, the retention of EAA was strongly influenced by dietary protein concentra-tion, with higher protein diets leading to enhanced retention of these critical micronutri-ents. This may suggest that a balanced intake of EAA is not only necessary for their depo-sition in larval biomass but also for maintaining optimal metabolic functions. Future re-search should further investigate these diet optimizations to standardize insect farming.

Conclusions

In this study, we assessed the effects of four different dietary crude protein levels on the growth performance, nutrient utilization, and retention of gross energy, nitrogen, and AA in BSFL. The highest larval performance was observed with 14% and 16% crude pro-tein (CP), while the 10% CP diet resulted in the lowest productivity. A 14% CP diet opti-mized nutrient utilization, leading to the highest levels of larval protein, nitrogen, and AA, except for cysteine. Our findings indicate that insufficient dietary protein levels limit larval protein biosynthesis and negatively impacts growth performance, body composition, and the retention efficiency of nitrogen and AA.

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Data Availability Statement: The datasets used and analyzed during the current study are availa-ble from the corresponding author on reasonable request.

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Abbreviations

AA: amino acids; BSFL: black soldier fly larvae; CP: crude protein; DM: dry matter; DOL: day-old-larvae; EAA: essential amino acids; FM: fresh matter; FCR: feed conversion ratio; GE: gross energy; GECR: gross energy conversion ratio; N: nitrogen; NCR: nitrogen conversion ratio; aNDFom: amylase neutral detergent fiber organic matter; NEAA: non-essential amino acids; SE: standard error; TAACR: total amino acid conversion ratio.

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5 Publication III

Performance and nutrient composition of black soldier fly larvae fed diets with various protein concentrations throughout the life cycle

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Abstract

Standardised rearing of black soldier fly (BSF, Hermetia illucens) larvae to utilise low-value organic substrates is gaining increasing interest in the context of sustainable animal production. The present study hypothesised that varying dietary protein concentrations would affect the growth performance and nutrient composition of BSF larvae across different life stages. To test this hypothesis, the effects of low (CPlow, 10% protein), medium (CPmed, 15% protein), and high (CPhigh, 20% protein) protein concentrations in isoenergetic diets were investigated on BSF growth from the juvenile to the adult stage, with six replicates per treatment. From day 10 (DOL, days-old larvae) onwards, larvae fed the CPlow diet had significantly lower live mass than those on CPmed and CPhigh diets, with reductions of 24 and 29% at 14 and 16 DOL, respectively, compared to CPmed-fed larvae ($P < 0.05$). This effect persisted through the prepupal and adult stages. Developmental time from egg to prepupa was shorter in the CPmed and CPhigh groups, resulting in larger prepupal and adult body sizes. Nutrient analysis revealed that larvae fed the CPmed diet had higher dry mass, protein, and amino acid mass at the prepupal stage compared to those on the CPlow diet, whereas fat deposition was increased in CPlow-fed larvae at certain developmental stages. This study demonstrates that both the nutrition of Hermetia illucens larvae and their life stage at separation are crucial for optimising their value as a feed source.

Keywords

Circular economy, Food by-products, *Hermetia illucens*, Innovative feed ingredients, Sustainability

Implications

Protein-rich feed ingredients are increasingly required in livestock production, yet conventional sources face sustainability challenges. This study confirms that dietary protein concentration significantly affects the nutrient profile of Hermetia illucens across its life stages. It supports the hypothesis that adjusting protein supply to the larvae's requirements improves growth and body composition. The findings apply to larvae reared under controlled laboratory conditions and emphasise that tailored dietary protein content enhances the nutritional value of black soldier fly larvae with high resource efficiency. These results are particularly relevant for researchers, insect farming industries, and feed manufacturers.

Introduction

The increasing global demand for protein feed components for livestock necessitates alternative sources to reduce reliance on soybean meal and fish meal. Insects offer a promising alternative due to their high protein content and their potential to convert organic low-value components into valuable feed (Gasco et al., 2023, van Huis and Gasco, 2023). In the European Union (EU), processed animal proteins from insects are approved for use in aquaculture, poultry, and pig feed (Veldkamp and Bosch, 2015). Increasing consumer acceptance further supports the potential of insect-based feed (Rossi et al., 2025, Menozzi et al., 2021). Insects contribute to sustainable livestock production by converting organic waste into high-quality protein and fat biomass, enhancing feed security with a reduced environmental impact compared to conventional protein sources.

Among edible insects, the black soldier fly (BSF, Hermetia illucens) is particularly effective in nutrient recycling and waste reduction. BSF larvae are rich in protein (∼40%) and fat (∼30%), making them a valuable feed ingredient for poultry (Stöhr et al., 2025a, Beller et al., 2024, Schäfer et al., 2023, Hartinger et al., 2022, Heuel et al., 2021, Dörper et al., 2021), pigs (Stöhr et al., 2025b), and aquaculture species (Gasco et al., 2025, Alvanou et al., 2023, Kumar et al., 2021, Stadtlander et al., 2017). They are also suitable for pet food production (Bosch and Swanson, 2021). Beyond animal feed, BSF larvae serve as a raw material for biofuel production (Surendra et al., 2016, Leong et al., 2016, Zheng et al., 2012), supporting waste-to-energy strategies.

The BSF life cycle includes five stages: egg, larva, prepupa, pupa, and adult (Li et al., 2011, Tomberlin et al., 2002). During the final larval stage, prepupae migrate to a dry pupation site

(Diener et al., 2011). Adult flies are neither pests nor disease vectors and survive on fat reserves from the larval stage, requiring only water (Banks et al., 2014).

Black soldier fly larvae can grow on nutrient-poor substrates (Klüber et al., 2024, Naser El Deen et al., 2023, Ribeiro et al., 2022, Gold et al., 2020, Spranghers et al., 2016), though higher dietary protein levels enhance their growth performance (Eggink et al., 2023, Bellezza Oddon et al., 2022). However, Barragan-Fonseca et al. (2021) and Schneider et al. (2025) demonstrated that an optimal dietary protein supply in BSF diets is more critical than simply increasing dietary protein levels. Insufficient protein levels (10% on DM basis) limited larval protein biosynthesis and negatively affected growth, body composition, and nitrogen and amino acid deposition (Schneider et al., 2025).

Liu et al. (2017) demonstrated that the biological development of BSF significantly influences the nutrient composition of larvae, which in turn affects their suitability as feed ingredients as well as the resource efficiency of nutrient conversion. The body composition of BSF changes with age, directly affecting the nutritional quality and digestibility of the insects when used as feed for poultry (Dörper et al., 2025) and pigs (Veldkamp and Vernooij, 2025). In addition, antinutritional factors such as chitin and ash increase as BSF age, impairing nutrient absorption (Oonincx and Finke, 2025). Therefore, optimising both dietary composition and the timing of larval separation is crucial for improving BSF conversion efficiency and the nutritional quality of the harvested insects.

Studies examining the effects of varying dietary protein concentrations on the growth performance and body composition of BSF at different life cycle stages remain limited. Based on the results of recent studies conducted in our laboratory and previous research (Schneider et al., 2025), we hypothesised that optimised dietary protein concentrations in isoenergetic diets would enhance growth performance, nutrient utilisation, and the nutritional value of BSF larvae throughout their life cycle. Therefore, the present study evaluated the effects of three isoenergetic experimental diets with differing protein concentrations on nutrient composition and performance across the developmental stages of BSF.

Material and methods

Experimental diets

Three isoenergetic diets with varying CP levels (10%, CPlow; 15%, CPmed; and 20%, CPhigh) were tested, with the standard Gainesville diet (Hogsette, 1992) serving as a positive control (Table 1). Before preparing the experimental diets, the DM content of all raw materials was determined using a precision moisture analyser (Radwag MA 200/1.X2.IC.A, Radon, Poland). The substrates were weighed and mixed with warm tap water (27 °C) to reach a moisture concentration of 75%. Following preparation, a 150 g representative sample was collected from each diet and analysed for nutrient concentrations to ensure the accuracy of the dietary composition (Table 5.1, Table 5.2).

Table 5.1: Ingredients and nutrient composition of the experimental diets fed to black soldier fly

Items	CON	CPlow	CPmed	CPhigh
Ingredients, g/kg				
Apple pomace	-	220.0	190.0	150.0
Rape seed cake	-	5.0	150.0	239.8
Wheat pulp	-	10.0	40.0	80.0
Biscuit flour	-	220.0	175.0	155.0
Bread crumbs	-	360.0	260.0	160.0
Rape seed oil	-	10.0	10.0	8.0
Wheat bran	500.0	175.0	175.0	175.0
Alfalfa	300.0	-	-	-
Maize	200.0	-	-	-
Analysed nutrient composition, g/kg DM [a]				
Dry matter	245	240	244	234
GE [MJ/kg]	16.3	18.3	18.5	18.4
Crude protein[b]	136	98	148	205
Crude ash	62.1	52.0	53.3	58.5
Ether extract	24.0	70.5	69.6	63.8
aNDFom	365	250	322	324
Starch	190	318	237	231
Calcium	4.1	2.9	3.2	3.4
Phosphorus	6.5	3.7	5.5	6.7

Dietary formulations for the control, CPlow, and CPhigh diets were partially adapted from Schneider et al. (2025).

Abbreviations: CON, control diet; CPlow, 10% CP diet; CPmed, 15% CP diet; CPhigh, 20% CP diet; GE, gross energy; aNDFom, amylase neutral detergent fiber organic matter.

[a] *Values are means of duplicate analyses*

[b] *Calculated as nitrogen × 6.25*

Figure 5.1: Analysed concentraamino acids in the experimental diets fed to black soldier fly

Items	CON	CPlow	CPmed	CPhigh
Total Amino acids, g/kg DM	135.6	91.8	142.5	195.6
	Essential amino acids composition, g/kg DM			
Arginine	7.9	4.5	7.6	10.3
Histidine	3.2	2.2	3.6	4.9
Isoleucine	5.5	3.6	5.6	7.8
Leucine	9.7	6.3	9.5	13.4
Lysine	6.7	3.7	6.2	9.0
Methionine	2.3	1.6	2.4	3.5
Phenylalanine	6.0	3.9	5.8	7.5
Threonine	5.2	3.4	5.9	8.1
Tryptophan	19.0	1.5	1.9	2.8
Valine	7.5	4.9	7.6	10.5

Abbreviations: CON, control diet; CPlow, 10% CP diet; CPmed, 15% CP diet; CPhigh, 20% CP diet

Experimental design and sample collection

The BSF larvae were obtained from a commercial breeding facility (madebymade GmbH, Pegau, Germany). After hatching, the larvae were initially reared on chicken feed until they reached 6 day-old-larvae (DOL) and transported in secure containers to the Department of Animal Nutrition at the University of Applied Sciences Bingen (TH Bingen). Rearing then continued in the laboratory of the Department of Animal Nutrition at the University of Applied Sciences, Bingen, Germany. To ensure uniformity in size, larvae were passed through sieves with mesh sizes of 2.4 and 1.0 mm to remove both undersized and oversized individuals. The average initial larval weight (7.0 ± 0.2 mg) was determined by manually counting the number of larvae in three 1 g samples (approximately 143 larvae per gram). This value was used to calculate the total number of larvae allocated to each experimental container as described by Schneider et al. (2025), 6 DOL were transferred to a controlled climate chamber (27 ± 1.5 °C and 55 ± 5% relative humidity) and randomly assigned to the respective treatment groups. Groups of 14 000 larvae were distributed in containers (40 × 60 × 12 cm; 2.1 cm^3/larva). Each container was filled with 2 800 g of dry substrate, corresponding to 0.2 g DM/larva and approximately 11 200 g fresh matter (FM) per container (0.8 g FM/larva). At beginning of the trial, the total larval weight per container was 100 g. The containers were arranged in a vertically stacked system and rotated daily to maintain homogeneous environmental conditions.

Samples were collected at multiple time points during the feeding and postfeeding stages. Sampling was conducted at 6, 8, 10, 12, 14, and 16 DOL. At each time point, 100 larvae per container were randomly sampled, washed, air−dried, weighed, and stored at −20 °C for analysis.

From the second day postprepupation onwards, 100 prepupae were sampled daily and stored at −20 °C. Pupation was monitored under controlled conditions (25 ± 1.5 °C, 65 ± 5% RH) with observations every 12 h for each replicate. Pupae were transferred to cages measuring 65 × 65 × 115 cm to monitor the timing of adult emergence. Once emergence was complete, late prepupae from the final days before pupation were collected, and adults were sampled 2 days after emergence. Male and female adults were stored separately in zip-lock bags at −20 °C for later analysis.

All samples were immediately stored at −20 °C and lyophilised within 36 h using a vacuum freeze-dryer (CHRIST, Osterode am Harz, Germany). After lyophilisation, samples were ground using a 1 mm sieve (ZM 100, RETSCH, GmbH, Haan, Germany) for further chemical analysis.

Analysis of diet and *Hermetia Illucens* composition

Samples were analysed using official methods of the Verband Deutscher Landwirt-schaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA, 2007). DM was determined by drying a 1 g sample at 103 °C for 4 h (method 3.1, VDLUFA, 2007). Nitrogen concentrations were measured using a nitrogen determinator (Leco Corporation, St. Joseph, MI, USA) by the Dumas method (VDLUFA, 2007). Amino acids were analysed by 24-h hydrolysis at 110 °C in 6 mol/L HCl, followed by HPLC (Waters Corporation, Milford, MA, USA) as described by Schneider et al. (2025).

Crude protein was calculated as nitrogen × 6.25. Larval protein was corrected for non-protein nitrogen using CPcor = N × 4.76 (Janssen et al., 2017). Ether extracts were determined gravimetrically after extracting the samples with petroleum ether using a Behr E6 system (method 5.11, VDLUFA, 2007). Ash content was determined by incineration at 550 °C for 6 h (method 8.1, VDLUFA, 2007). NDF was determined using α-amylase (Fibertherm FT 12, Gerhardt, Germany) and expressed without residual ash (methods 6.5.1 and 6.5.2, VDLUFA, 2007). Calcium and phosphorus were measured by ICP-OES (Quantima, GBC Scientific Equipment, Australia) (method 10.8.2, VDLUFA, 2007). Starch was analysed by colorimetry (Roche Cobas Mira S; Randox GLUC-PAP Kit) after enzymatic hydrolysis (Merck, 10115-5G-F, Darmstadt, Germany) (method 7.2.1, VDLUFA, 2007).

Determination of chitin

The chitin/chitosan content in the insect samples was quantified using the method by Urs et al. (2023) as described in Schneider et al. (2025). Five mg of freeze-dried samples was suspended in 0.5 mL of 6% KOH, incubated at 80 °C for 90 min, and centrifuged to remove KOH. The pellet was washed twice with phosphate-buffered saline and homogenised in 250 µL of water. To convert glucosamine into N-acetylglucosamine, the pH was adjusted to ~8.5 with 1 M $NaHCO_3$, followed by the addition of 50 µL of $[^2H_6]$acetic anhydride. Then, fully acetylated sample was enzymatically hydrolysed into GlcNAc and $[^2H_3]$GlcNAc monomers by adding 0.1 µg of the chitinase ChiB, 0.1 µg of the chitosanase CSN-174, and 0.1 units of *Trichoderma viride* chitinase (Sigma-Aldrich, St-Louis, USA). After incubation at 30 °C for 48 h under shaking, the samples were freeze-dried, resuspended in water, filtered, and mixed with a double isotopically labelled internal standard for LC-MS quantification of the chitinous material in the sample.

Calculations

Live mass was determined from a sample of 100 randomly selected individuals per box, with six replicate boxes per treatment. The larval survival rate was determined by calculating the ratio of the prepupa count to the initially estimated number of BSFL. Prepupa total biomass gain was measured as the difference between the total prepupa mass per container and the initial larval mass. Substrate reduction was calculated by subtracting the remaining substrate weight at the prepupal stage from the initial substrate weight per container. To calculate the time required for larvae to progress from egg to prepupa and from prepupa to adult, the developmental durations were determined by monitoring the age of individuals at each developmental stage. The time from egg to prepupa was calculated as the difference between the day of prepupal appearance and the day of egg deposition. Similarly, the time from prepupa to adult was calculated as the difference between the day of adult emergence and the day prepupation began. These time intervals were averaged across replicates to obtain the mean duration for each developmental phase. The efficiency of development was assessed by comparing the time required for larvae to transition through each stage under different rearing conditions. The body length of BSF prepupa and adults was measured using a digital calliper (accuracy: 0.01 mm), with larval length taken from the head capsule to the last segment and adult body length from the head to the tip of the abdomen.

Statistical analysis

The rearing container constituted the experimental unit, with six replicates per dietary treatment. Statistical analyses were performed using IBM SPSS Statistics (version 29). ANOVA was applied, followed by Tukey's HSD posthoc test for multiple comparisons among dietary groups. The dataset included larval weight, developmental duration, body size, and chemical composition. The normal distribution of the data was tested using the Shapiro-Wilk test, and the homogeneity of variances was evaluated with Levene's test. Normal distribution was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated with Levene's test. If data deviated from the normal distribution, a logarithmic transformation was applied, and the transformed values were used for statistical evaluation. Further, a curve-fitting linear and non−linear regression analysis was performed. Statistical significance was determined at $P \leq 0.05$, and results are reported as means ± SD. The control diet was not included in the statistical analysis for comparisons with test diets.

Results

Growth performance of *Hermetia illucens*

No differences in live mass were observed at 6 DOL. However, from 10 DOL onwards, significant effects of dietary protein levels became evident. At 10 DOL, larvae fed the CPlow diet had a lower live mass (100 mg) compared to those fed the CPmed (129 mg) and CPhigh (140 mg) diets ($P < 0.05$; Fig. 5.1 A). This trend continued throughout development: at 12 DOL, larvae of the CPlow group reached 137 mg, compared to 186 mg in the CPmed group; at 14 DOL, 153 mg versus 210 mg, respectively. By 16 DOL, live mass of CPlow-fed larvae was 160 mg, compared to 207 mg in the CPmed group.

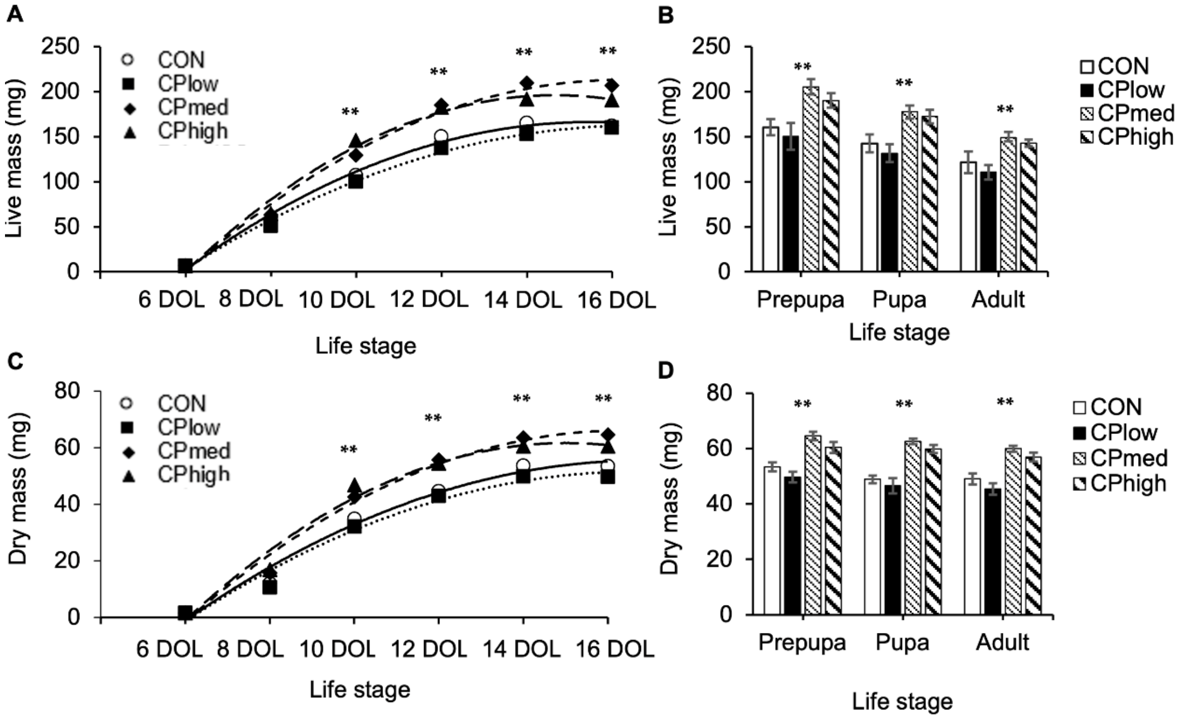

*Figure 5.2: Growth performance of black soldier fly at different life stages under varying dietary protein levels. (A, C) Live mass and (B, D) dry mass of black soldier fly over time. (A, B) Growth trends of larvae from 6 to 16 days of life (DOL). (C, D) Mass at the prepupal, pupal, and adult stages. Data are presented as means (n = 6/diet). CON was not included in the statistical analysis. Dietary groups: CON = control diet; CPlow = low protein diet; CPmed = medium protein diet; CPhigh = high protein diet. Data were fitted using a second-order polynomial function. Significant differences between dietary groups at each time point are indicated by **$P < 0.01$.*

At the prepupal stage, larvae of the CPlow group had a live mass of 151 mg, while larvae of the CPmed and CPhigh groups reached 205 mg and 191 mg, respectively ($P < 0.05$; Fig. 5.1B). This effect was also evident at the pupal and adult stages: pupae from the CPlow group reached 132 mg, compared to 177 mg in the CPmed group; adults reached 110 mg and 149 mg, respectively ($P < 0.05$; Fig. 5.1B).

The dry mass of BSF increased with age, peaking at the prepupal stage before slightly declining. BSF of the medium- and high-protein groups exhibited significantly higher dry mass than BSF of the low-protein group at 10–14 days of larval age and in the prepupal and adult stages ($P < 0.05$, Fig. 5.1C and D).

Development time and body size of *Hermetia illucens*

Larvae developed more rapidly with increasing dietary protein concentration. The time from 6 DOL to prepupa was significantly shorter in the high-protein group compared to the low-protein group ($P < 0.05$, Table 5.3), while the medium-protein group showed intermediate values. Similarly, the prepupa-to-adult transition was significantly longer in the low-protein group compared to all other groups ($P < 0.05$, Table 5.3). Consequently, the total 6 DOL-to-adult developmental time (Table 5.3) was reduced in the CPmed and CPhigh groups compared to the CPlow group ($P < 0.05$).

Table 5.2: Development and performance parameters of black soldier fly in different life stages

Items	CON	CPlow	CPmed	CPhigh
Survival rate prepupa, %	94.2 ± 0.3	91.1 ± 0.4	94.6 ± 0.2	93.0 ± 0.4
Total biomass gain [1], kg FM	1.73 ± 0.1	1.66 ± 0.2^{a}	2.31 ± 0.2^{a}	2.20 ± 0.1^{a}
Substrate reduction [2], kg FM	3.15 ± 0.3	3.66 ± 0.3a	3.60 ± 0.2^{a}	2.82 ± 0.4^{b}
6 DOL-to-prepupa, d	10.2 ± 1.0	10.9 ± 0.4^{a}	9.7 ± 0.5^{ab}	9.2 ± 0.4^{b}
Prepupae-to-adult, d	11.0 ± 0.8	12.4 ± 0.4^{a}	11.2 ± 0.5^{b}	11.0 ± 0.3^{b}
6 DOL-to-adult, d	21.2 ± 1.0	23.3 ± 0.3^{a}	20.9 ± 0.4b	20.2 ± 0.3b
Body length prepupa, mm	11.0 ± 0.1	10.7 ± 0.6^{a}	13.3 ± 1.2a	12.8 ± 0.8^{a}
Body length female, mm	13.1 ± 0.2	13.6 ± 0.2b	15.1 ± 1.1^{a}	14.8 ± 1.0^{a}
Body length male, mm	12.7 ± 0.1	13.1 ± 0.2b	14.2 ± 0.2^{a}	14.1 ± 0.7^{a}

Abbreviations: CON, control diet; CPlow, 10% CP diet; CPmed, 15% CP diet; CPhigh, 20% CP diet; FM, fresh matter; DOL, day-old-larvae

$^{a-b}$*: Values in a row that are marked without the same superscript letter differ significantly ($P < 0.05$)*

Data are presented as means (n = 6 boxes/diet) and standard deviation (SEM)

[1] *Calculated as prepupa weight per container (kg)—initial weight per container (kg).* [2] *Calculated as substrate weight at prepupa stage per container (kg)—initial substrate weight per container (kg).*

At the prepupal stage, survival rates did not differ significantly between dietary treatments. Total biomass gain was significantly higher in larvae fed CPmed and CPhigh diets compared to CPlow ($P < 0.05$, Table 5.3). Substrate reduction was highest in the CPlow and CPmed groups, while significantly lower values were observed in the CPhigh group ($P < 0.05$, Table 5.3).

Prepupal body size was significantly larger in the medium- and high-protein groups compared to the low-protein group ($P < 0.05$, Table 5.3). Among adults, both females and males in these groups attained significantly larger body lengths than those in the low-protein group ($P < 0.05$, Table 5.3), while the control group exhibited intermediate values.

Nutrient composition of *Hermetia illucens*

Protein mass increased continuously from 6 to 16 DOL across all dietary groups, reaching the highest values at the prepupal stage. Larvae in the CPmed and CPhigh groups exhibited significantly greater protein mass than those in the CPlow and control diet (CON) groups starting from 8 DOL ($P < 0.05$, Table 5.4). The CON group showed intermediate values between CPlow and CPmed.

Table 5.3: Masses of nutrients of black soldier fly at different life stages

	Life stage							
	6 DOL	8 DOL	10 DOL	12 DOL	14 DOL	Prepupa	Pupa	Adult
Protein mass[1], g DM								
CON	0.5 ± 0.0	5.7 ± 0.2	11.5 ± 0.2	18.6 ± 0.7	19.5 ± 1.0	21.4 ± 0.4	23.9 ± 0.8	19.7 ± 0.9
CPlow	0.5 ± 0.0^{a}	3.1 ± 0.3^{a}	11.2 ± 0.6^{b}	14.3 ± 0.5^{b}	15.9 ± 0.5^{b}	17.3 ± 0.6^{b}	19.8 ± 0.8^{b}	16.0 ± 0.4^{b}
CPmed	0.5 ± 0.0^{a}	4.5 ± 0.4^{a}	15.7 ± 0.8^{a}	19.5 ± 0.6^{a}	23.1 ± 0.5^{a}	23.3 ± 0.4^{a}	25.7 ± 0.4^{a}	23.0 ± 1.5^{a}
CPhigh	0.5 ± 0.0^{a}	4.4 ± 0.4^{a}	17.0 ± 1.1^{a}	18.9 ± 0.9^{a}	20.7 ± 0.7^{a}	22.5 ± 0.7^{a}	26.3 ± 0.9^{a}	25.1 ± 0.8^{a}
Fat mass[1], g DM								
CON	0.1 ± 0.0	2.5 ± 0.1	5.8 ± 0.2	10.5 ± 0.6	12.9 ± 0.9	13.3 ± 0.3	9.9 ± 0.4	6.3 ± 0.5
CPlow	0.1 ± 0.0^{a}	2.2 ± 0.2^{a}	8.6 ± 0.4^{a}	13.5 ± 0.6^{a}	18.2 ± 0.5^{a}	19.1 ± 0.9^{a}	17.6 ± 0.6^{a}	11.5 ± 0.3^{a}
CPmed	0.1 ± 0.0^{a}	2.4 ± 0.2^{a}	7.8 ± 0.4^{a}	12.4 ± 0.2^{b}	15.0 ± 0.2^{b}	17.4 ± 0.3^{b}	16.1 ± 0.6^{b}	8.4 ± 0.2^{b}
CPhigh	0.1 ± 0.0^{a}	2.6 ± 0.2^{a}	9.2 ± 0.7^{a}	13.0 ± 0.5^{ab}	16.2 ± 0.6^{ab}	17.6 ± 0.4^{b}	16.6 ± 0.5^{b}	7.6 ± 0.3^{b}
Chitin mass[1], g DM								
CON	0.1 ± 0.0	0.7 ± 0.1	2.1 ± 0.2	2.4 ± 0.3	2.7 ± 0.2	4.6 ± 0.3	5.0 ± 0.4	5.4 ± 0.2
CPlow	0.1 ± 0.0^{a}	0.4 ± 0.1^{a}	2.0 ± 0.2^{b}	2.2 ± 0.3^{b}	2.5 ± 0.3^{b}	3.9 ± 0.2^{b}	4.7 ± 0.6^{b}	5.0 ± 0.4^{b}
CPmed	0.1 ± 0.0^{a}	0.4 ± 0.0^{a}	2.1 ± 0.1^{b}	2.8 ± 0.1^{a}	3.1 ± 0.4^{a}	4.9 ± 0.4^{a}	5.4 ± 0.2^{a}	5.7 ± 0.4^{a}
CPhigh	0.1 ± 0.0^{a}	0.7 ± 0.1^{a}	3.6 ± 0.2^{a}	3.1 ± 0.2^{a}	3.4 ± 0.3^{a}	5.2 ± 0.9^{a}	5.5 ± 0.3^{a}	5.8 ± 0.3^{a}
Ash mass[1], g DM								
CON	0.1 ± 0.0	1.3 ± 0.1	2.8 ± 0.1	5.1 ± 0.2	5.0 ± 0.2	6.4 ± 0.3	6.6 ± 0.1	6.0 ± 0.9
CPlow	0.1 ± 0.0^{a}	0.6 ± 0.1^{a}	2.2 ± 0.1^{b}	3.1 ± 0.1^{b}	3.4 ± 0.1^{a}	6.0 ± 0.3^{b}	5.6 ± 0.5^{b}	5.2 ± 0.5^{a}
CPmed	0.1 ± 0.0^{a}	0.7 ± 0.1^{a}	2.8 ± 0.2^{ab}	3.9 ± 0.1^{ab}	4.2 ± 0.1^{ab}	7.4 ± 0.2^{a}	7.3 ± 0.3^{a}	6.5 ± 0.5^{a}
CPhigh	0.1 ± 0.0^{a}	0.9 ± 0.1^{a}	4.0 ± 0.2^{a}	4.9 ± 0.1^{a}	4.5 ± 0.1^{a}	7.5 ± 0.3^{a}	7.1 ± 0.2^{a}	6.2 ± 0.3^{a}

Abbreviations: CON, control diet; CPlow, 10% CP diet; CPmed, 15% CP diet; CPhigh, 20% CP diet; DOL, day-old-larvae

[1] Calculated as nutrient concentration (mg/kg on DM basis) in a sample of 100 individuals $^{a\text{-}b}$: Values in a row that are marked without the same superscript letter differ significantly ($P < 0.05$). Data are presented as means ($n = 6$ boxes/diet) and standard deviation (SEM)

The ether extract mass increased during early development, peaking at the prepupal stage before slightly declining in the adult stage. The CPlow group showed significantly lower ether extract mass than the CPmed and CPhigh groups ($P < 0.05$, Table 5.4), while the CON group showed lower values than other groups.

Chitin mass increased steadily throughout development, with the highest values observed in the pupal stage. Chitin mass was significantly higher in the CPmed and CPhigh groups than in the CPlow group at 10 DOL and beyond ($P < 0.05$, Table 5.4). The CON group showed intermediate chitin mass compared to the protein-supplemented groups.

Crude ash mass followed a similar trend, increasing during larval development and peaking at the prepupal and pupal stages. The CPmed and CPhigh groups showed significantly higher crude ash mass than the CPlow from 10 DOL onwards ($P < 0.05$, Table 5.4). The CON group exhibited higher crude ash mass than the CPlow group at 12 DOL and beyond.

Dynamic changes of calcium, phosphorus, and amino acid masses

The calcium mass of BSF fed with different diets increased throughout the developmental stages, with a peak at the pupal stage and a decrease in the adult stage. BSF fed with the medium- and high-protein diets showed significantly higher calcium mass than BSF fed with the low-protein diet at 10–14 days of larval age in the prepupal, pupal, and adult stages ($P < 0.05$, Table 5.5).

Table 5.4: Calcium and phosphorus mass and Ca/P-ratio of black soldier fly in different life stages.

	Life stage							
	6 DOL	8 DOL	10 DOL	12 DOL	14 DOL	Prepupa	Pupa	Adult
Calcium mass[1], mg DM								
CON	26.4 ± 1.1	259 ± 59.2	514 ± 36.4	929 ± 63.1	1070 ± 44.2	1186 ± 93.2	1143 ± 78.2	400 ± 28.2
CPlow	25.6 ± 1.0^{a}	121 ± 13.8^{a}	350 ± 31.9^{c}	498 ± 32.9^{b}	611 ± 41.5^{b}	671 ± 33.6^{b}	708 ± 42.3^{a}	239 ± 8.2^{b}
CPmed	25.3 ± 1.0^{a}	163 ± 10.5^{a}	456 ± 33.8^{b}	652 ± 30.7^{a}	858 ± 40.4^{a}	902 ± 47.6^{a}	974 ± 73.2^{b}	310 ± 16.3^{a}
CPhigh	25.2 ± 0.9^{a}	199 ± 16.6^{a}	604 ± 48.1^{a}	749 ± 47.7^{a}	914 ± 51.8^{a}	969 ± 53.7^{a}	1173 ± 62.0^{b}	358 ± 19.3^{a}
Phosphorus mass[1], mg DM								
CON	6.9 ± 0.6	108 ± 7.9	228 ± 14.0	403 ± 22.7	482 ± 39.8	550 ± 33.4	501 ± 40.8	650 ± 37.4
CPlow	6.5 ± 0.5 ^{a}	47 ± 4.0^{a}	184 ± 11.0^{b}	258 ± 17.2^{b}	324 ± 16.5^{b}	375 ± 19.0^{b}	373 ± 15.8^{b}	468 ± 20.9^{b}
CPmed	6.6 ± 0.6 ^{a}	39 ± 3.9^{a}	176 ± 17.0^{b}	287 ± 20.4^{a}	411 ± 10.2^{a}	469 ± 17.2^{a}	521 ± 19.1^{a}	566 ± 15.0^{a}
CPhigh	6.4 ± 0.5 ^{a}	65 ± 5.2^{a}	267 ± 17.1^{a}	339 ± 17.2^{a}	405 ± 19.6^{a}	450 ± 21.4^{a}	516 ± 26.1^{a}	583 ± 29.3^{a}

	Life stage							
	6 DOL	8 DOL	10 DOL	12 DOL	14 DOL	Prepupa	Pupa	Adult
Ca/P-ratio, g/g DM								
CON	3.8 ± 0.0	2.4 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.2 ± 0.2	2.2 ± 0.3	2.3 ± 0.1	0.6 ±0.1
CPlow	3.9 ± 0.1 ^{a}	2.6 ± 0.1^{b}	1.9 ± 0.2^{b}	1.9 ± 0.3^{a}	1.9 ± 0.0^{a}	1.8 ± 0.1^{a}	1.9 ± 0.1^{a}	0.5 ± 0.0^{a}
CPmed	3.8 ± 0.1 ^{a}	4.2 ± 0.0^{a}	2.6 ± 0.1^{a}	2.3 ± 0.1^{a}	2.1 ±0.1^{a}	1.9 ± 0.0^{a}	1.9 ± 0.2^{a}	0.6 ± 0.0^{a}
CPhigh	3.9 ± 0.1 ^{a}	3.1 ± 0.3^{ab}	2.3 ± 0.1^{ab}	2.2 ± 0.2^{a}	2.3 ± 0.1^{a}	2.1 ± 0.1^{a}	2.3 ± 0.3^{a}	0.6 ± 0.1^{a}

Abbreviations: CON, control diet; CPlow, 10% CP diet; CPmed, 15% CP diet; CPhigh, 20% CP diet; DM, dry matter; DOL, day-old-larvae
[1] *Calculated as nutrient concentration (mg/kg on DM basis) in a sample of 100 individuals*
$^{a-c}$*: Values in a row that are marked without the same superscript letter differ significantly (P < 0.05)*
Data are presented as means (n = 6 boxes/diet) and standard deviation (SEM)

In contrast, phosphorus concentrations increased consistently over the course of development, but no significant differences were observed between the dietary groups at any life stage.

The calcium-to-phosphorus (Ca/P) ratio was highest in BSF fed with the low-protein group at 6–8 days of larval age. BSF fed with the medium- and high-protein diets had significantly higher Ca/P ratios compared to BSF of the low-protein group at 10–14 days of larval age and in the prepupal and pupal stages (P < 0.05, Table 5.5). Thus, the Ca/P ratio showed a decreasing trend with age, particularly for BSF fed with the low-protein diet. Overall, amino acid masses increase with age across all dietary treatments, with BSF fed higher protein content diets (CPmed and CPhigh) generally showing higher amino acid concentrations than BSF fed the lower protein diet (CPlow). Particularly in the adult stage, clear differences between dietary groups are observed. Statistical analysis (P < 0.05, Table 5.6) reveals significant differences between the diets, with the higher protein diets consistently showing significantly higher amino acid masses compared to the low-protein diet.

Table 5.5: Amino acid mass of black soldier fly in different life stages.

	Life stage							
	6 DOL	8 DOL	10 DOL	12 DOL	14 DOL	Prepupa	Pupa	Adult
Total amino acid mass^{1}, g DM								
CON	0.4 ± 0.0	3.7 ± 0.1	8.2 ± 0.4	13.5 ± 0.3	15.4 ± 0.2	16.8 ± 0.5	9.8 ± 0.2	18.7 ± 0.2
CPlow	0.3 ± 0.1^{a}	2.1 ± 0.2^{b}	8.3 ± 0.6^{b}	10.9 ± 0.4^{b}	13.6 ± 0.6^{b}	15.1 ±0.4^{b}	9.8 ±0.6^{b}	18.3 ± 0.3^{b}
CPmed	0.4 ± 0.0^{a}	3.0 ± 0.3^{a}	11.1 ± 0.5^{a}	14.5 ±0.5^{a}	18.5 ± 0.5^{a}	19.5 ±0.4^{a}	12.8 ±0.5^{a}	24.6 ± 0.5^{a}
CPhigh	0.4 ± 0.1^{a}	3.0 ± 0.3^{a}	12.3 ± 0.6^{a}	13.6 ± 0.6^{a}	16.5 ± 0.6^{a}	17.8 ±0.3^{a}	12.3 ±0.6^{a}	23.4 ± 0.6^{a}
Isoleucine mass^{1}, mg DM								
CON	21.0 ± 0.2	228 ± 9.0	443 ± 3.4	731 ± 19.7	795 ± 35.4	864 ± 17.8	645 ± 14.2	971 ± 38.0
CPlow	20.0 ± 0.04^{a}	126 ± 11.1^{a}	435 ± 23.2^{b}	567 ± 24.3^{b}	652 ± 25.6^{b}	713 ± 26.9^{b}	581 ± 22.0^{b}	924 ± 32.2^{b}
CPmed	20.3 ± 0.03^{a}	181 ± 14.6^{a}	585 ± 30.8^{a}	767 ± 21.6^{a}	944 ± 20.6^{a}	981 ± 15.9^{a}	692 ± 34.4^{a}	1246 ± 27.4^{a}
CPhigh	20.2 ± 0.04^{a}	177 ± 13.3^{a}	657 ± 42.2^{a}	745 ± 0.04^{a}	848 ± 33.6^{a}	892 ± 34.4^{ab}	758 ± 16.2^{a}	1190 ± 49.1^{a}
Leucine mass^{1}, mg DM								
CON	31.4 ± 0.4	368 ±13.7	794 ± 19.6	1290 ± 47.6	1440 ± 77.2	1564 ± 55.9	981 ± 44.0	1730 ± 51.1
CPlow	30.2 ± 0.6^{a}	206 ± 19.0^{a}	812 ± 45.2^{b}	1045 ± 43.7^{b}	1231 ± 51.3^{b}	1348 ± 34.2^{b}	958 ± 31.6^{a}	1515 ± 78.0^{b}
CPmed	31.0 ± 0.2^{a}	291 ± 22.7^{a}	1050 ± 52.9^{a}	1357 ± 36.2^{a}	1712 ± 31.2^{a}	1783 ± 39.4^{a}	1177 ± 55.3^{a}	2223 ± 43.7^{a}
CPhigh	30.4 ± 0.3^{a}	289 ± 24.4^{a}	1207 ± 78.1^{a}	1331 ± 40.0^{a}	1557 ± 68.0^{a}	1639 ± 69.4^{a}	1205 ± 46.5^{a}	1998 ± 87.2^{a}
Lysine mass^{1}, mg DM								
CON	25.7 ± 0.8	242 ± 7.2	603 ± 7.1	1032 ± 30.2	1136 ± 45.0	1253 ± 20.0	461 ± 28.5	1577 ± 71.9
CPlow	23.3 ± 0.3^{a}	135 ± 14.7^{a}	545 ± 29.2^{b}	749 ± 30.6^{b}	857 ± 30.0^{b}	956 ± 40.7^{b}	497 ± 13.6^{a}	1407 ± 55.0^{b}
CPmed	24.0 ± 0.3^{a}	212 ± 15.3^{a}	785 ± 36.5^{a}	1077 ± 38.0^{a}	1332 ± 21.9^{a}	1344 ± 19.8^{a}	465 ± 23.0^{a}	1927 ± 32.4^{a}
CPhigh	24.1 ± 0.5^{a}	208 ± 10.3^{a}	884 ± 70.7^{a}	1088 ± 63.0^{a}	1202 ± 30.7^{a}	1216 ± 47.4^{a}	544 ± 17.7^{a}	1944 ± 53.8^{a}
Methionine mass^{1}, mg DM								
CON	7.8 ± 0.2	78.7 ± 2.7	197 ± 2.1	308 ± 9.7	329 ± 14.5	350 ± 9.1	203 ± 7.1	492 ± 20.7
CPlow	7.5 ± 0.1^{a}	41.2 ± 3.8^{a}	180 ± 9.0^{b}	233 ± 10.2^{b}	263 ± 9.4^{b}	282 ± 9.0^{b}	180 ± 4.8^{b}	451 ± 6.9^{b}
CPmed	7.4 ± 0.2^{a}	62.2 ± 5.1^{a}	248± 13.2^{a}	324 ± 9.7^{a}	390 ± 9.3^{a}	398 ± 7.2^{a}	259 ± 9.5^{a}	620 ± 11.7^{a}
CPhigh	7.6 ± 0.1^{a}	59.6 ± 4.8^{a}	273 ± 19.7^{a}	309 ± 18.6^{a}	345 ± 19.4^{a}	357 ± 20.1^{ab}	262 ± 14.6^{a}	610 ± 13.8^{a}
Threonine mass^{1}, mg DM								
CON	16.5 ± 0.1	185 ± 5.5	398 ± 3.1	676 ± 15.7	720 ± 26.1	775 ± 17.0	464 ± 16.2	1002 ± 57.7
CPlow	15.9 ± 0.3^{a}	96.3 ± 8.8^{a}	379 ± 23.3^{b}	509 ± 22.9^{b}	574 ± 28.1^{b}	622 ± 30.8^{b}	408 ± 30.7^{b}	778 ± 33.0^{b}
CPmed	16.0 ±0.2^{a}	141 ± 11.0^{a}	509 ± 23.6^{a}	688 ± 109^{a}	831 ± 19.7^{a}	856 ± 17.8^{a}	574 ± 23.2^{a}	1090 ± 52.3^{b}
CPhigh	16.4 ± 0.4^{a}	138 ± 9.8^{a}	570 ± 31.7^{a}	665 ± 27.0^{a}	745 ± 25.6^{a}	776 ± 27.6^{ab}	588 ± 21.5^{a}	1005 ± 43.1^{b}
Tryptophan mass^{1}, mg DM								
CON	10.2 ± 0.1	100 ± 3.4	218 ± 3.5	371 ± 12.0	404 ± 20.0	425 ± 10.7	323 ± 31.2	383 ± 26.0
CPlow	10.0 ± 0.1^{a}	50.9 ± 4.3^{a}	203 ± 12.0^{b}	273 ± 14.3^{b}	314 ± 15.1^{b}	334 ± 15.0^{b}	290 ± 15.0^{b}	315 ± 10.6^{b}
CPmed	9.8 ± 0.2^{a}	84.4 ± 6.2^{a}	318 ± 16.0^{a}	415 ± 8.9^{a}	512 ± 8.3^{a}	516 ± 5.4^{a}	416 ± 15.7^{a}	418 ± 7.9^{a}
CPhigh	10.2 ± 0.2^{a}	85.7 ± 7.0^{a}	357 ± 21.8^{a}	410 ± 18.4^{a}	474 ± 16.4^{a}	584 ± 16.0^{a}	493 ± 38.7^{a}	420 ± 14.5^{a}
Valine mass^{1}, mg DM								
CON	30.4 ± 0.3	288 ± 9.4	627 ± 6.4	1104 ± 44.0	1147 ± 51.1	1220 ± 20.5	1104 ± 84.8	1310 ± 32.8
CPlow	29.3 ± 0.2^{a}	151 ± 13.5^{a}	602 ± 31.5^{a}	844 ± 40.6^{a}	921 ± 36.2^{a}	988± 37.2^{a}	1012 ± 35.0^{a}	1118 ± 36.7^{a}

	Life stage							
	6 DOL	8 DOL	10 DOL	12 DOL	14 DOL	Prepupa	Pupa	Adult
CPmed	30.1 ± 0.4^{a}	227 ± 17.2^{a}	824 ± 37.3^{b}	1150 ± 34.7^{b}	1360 ± 23.5^{b}	1386 ± 21.1^{b}	1369 ± 96.0^{b}	1630 ± 9.0^{b}
CPhigh	30.3 ± 0.6^{a}	220 ± 17.6^{a}	925 ± 56.1^{b}	1108 ± 58.0^{b}	1221 ± 47.9^{b}	1259 ± 49.1^{a}	1537 ± 43.3^{b}	1526 ± 9.5^{b}

Abbreviations: CON, control diet; CPlow, 10% CP diet; CPmed, 15% CP diet; CPhigh, 20% CP diet; FM, fresh matter, DM, dry matter; DOL, day-old-larvae

[1] *Calculated as nutrient concentration (mg/kg on DM basis) in a sample of 100 individuals*

$^{a-b}$*: Values in a row that are marked without the same superscript letter differ significantly ($p < 0.05$)*

Data are presented as means (n = 6 boxes/diet) and standard deviation (SEM)

The amino acid masses (e.g., arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) varied depending on both life stage and diet. Notably, BSF larvae reared on the CPmed and CPhigh diets consistently exhibited higher amino acid concentrations compared to those fed the CPlow diet across most developmental stages. However, no significant differences in amino acid masses were observed between the dietary groups at the pupal stage (Table 5.6).

Discussion

Edible insects, especially BSF, have gained attention as a sustainable alternative to traditional feed sources for poultry (Stöhr et al., 2025a, Beller et al., 2024, Schäfer et al., 2023, Hartinger et al., 2022, Heuel et al., 2021, Dörper et al., 2021), pigs (Stöhr et al, 2025b), aquaculture (Gasco et al., 2025, Alvanou et al., 2023, Kumar et al., 2021, Stadtlander et al., 2017), pets (Bosch and Swanson, 2021) as well as biotechnological applications (Schäfer et al., 2025, Tettamanti and Bruno, 2024, Surendra et al., 2016). Previous studies have focused on the growth performance and changes in nutrient composition of BSF larvae and prepupae, depending on the heterogeneous feed provided. However, this study offers new insights into the effects of dietary protein on performance and the nutritional composition across the full life cycle, from the juvenile to the adult stage of BSF.

Effects of dietary protein concentration on performance

In this study, dietary protein concentrations of 15% (CPmed), with a gross energy content of 18.5 ± 0.3 MJ/kg DM, were identified as optimal for maximised growth of BSF. These findings underscore the critical role of dietary protein in the development of BSF during different life stages, corroborating previous research (Schneider et al., 2025, Barragan-Fonseca et al., 2021). BSF larvae can grow on nutrient-poor substrates (Klüber et al., 2024, Naser El Deen et al., 2023, Ribeiro et al., 2022, Gold et al., 2020, Spranghers et al., 2016). Higher dietary protein levels enhance growth performance (Eggink et al., 2023, Bellezza Oddon et al., 2022). However, Barragan-Fonseca et al. (2021) and Schneider et al. (2025) demonstrated that providing an optimal protein-to-energy ratio, rather than simply increasing dietary protein levels, is more critical for performance. In agreement with the present findings, Bellezza Oddon et al. (2022) reported that protein levels between 14 and 16% optimise larval development. As discussed in previous studies (Schneider et al., 2025), excessive dietary protein intake in Hermetia illucens larvae may lead to elevated mortality, attributed to metabolic stress, oxidative damage, and the accumulation of toxic catabolites (Tschirner and Simon, 2015, Eggink et al., 2023, Lee et al., 2008). In dipteran species, surplus uric acid is converted to ammonium, contributing to nitrogen emissions (Green and Popa, 2012). Larval growth and development are regulated by IGF and TOR signalling pathways, alongside ecdysteroids and juvenile hormones (Tomberlin et al., 2023, Nguyen et al., 2013, Mirth and Riddiford, 2007), which may be disrupted by excess protein intake. Nguyen et al. (2013) further highlighted that high protein levels can reduce feed intake due to increased metabolic load. In our study, larvae fed a 20% CP diet exhibited lower feed intake, likely restricting growth. Extended developmental times were associated with low-protein, high-carbohydrate diets, consistent with earlier findings (Cammack and Tomberlin, 2017, Barragan-Fonseca et al., 2019). These results highlight the need for precise macronutrient balancing to optimise larval performance and reduce nitrogen losses.

In this study, a correlation was observed between larval size and the size of the adult flies. Prepupa that exhibited larger body length during development resulted in adult flies that were also larger. Similarly, other studies have also shown that mating success and reproductive output can be influenced by adult body size in BSF and other insect species. For instance, larger males have demonstrated higher mating success and greater competitive ability, while larger females tend to produce more eggs (Jones and Tomberlin, 2020, Kaspi et al., 2002). This suggests that the relationship between larval size and egg production may reflect an enhanced physiological capacity in the flies, enabling them to invest more energy into reproduction, which could in turn optimise population dynamics and the efficiency of BSF breeding programmes. Our results suggest that a diet with 15% protein content and an energy content of 18.5 MJ/kg DM may offer an optimal nutrient supply, fulfilling the larvae’s nutritional needs without causing inefficiencies linked to excessive protein intake. Adequate protein levels are essential for supporting efficient protein biosynthesis — required for survival, growth, development, and reproduction, including enzymes, structural proteins, transport proteins, immune proteins, and hormones (Lemme and Klüber, 2024, Tomberlin et al., 2023, Nguyen et al., 2013, Dabour et al., 2011, Mirth and Riddiford, 2007, Oldham and Hafen, 2003). This highlights the importance of providing an adequate dietary protein supply to enhance larval growth for insect biomass production and to ensure healthy adults for reproduction.

Life stage effects on nutrient contents

The nutrient composition of BSF is highly dynamic and influenced by their life cycle stage and biological age. Our study demonstrated that protein, fat, chitin, ash, minerals, and amino acid concentrations in BSF undergo significant changes throughout insect development. The protein content is relatively high in the early juvenile larval stages, supporting rapid tissue growth and metabolic activity. Larvae fed a 15% protein diet (CPmed) showed higher protein deposition than those on lower protein diets. Amino acid profiles varied across developmental stages, with higher concentrations of essential amino acids in the early larval stages. This may reflect the higher demand for essential amino acids for protein synthesis during rapid juvenile growth. As larvae progressed to the prepupal stage, protein content decreased slightly while fat content increased, reflecting the metabolic shift towards energy storage required for metamorphosis. Fat stores are usually highest in the final larval or nymphal stage (Oonincx and Finke, 2025, Fast, 1970). Oonincx and Finke (2025) reported that this pattern depends to some extent on whether the species is holometabolous or hemimetabolous. In holometabolous species, larvae exhibit a higher fat concentration compared to adults (Lease and Wolf, 2011). For instance, the fat content in BSF (Liu et al., 2017) increases as they develop but drops significantly in the adult stage because fat is used as an energy source during pupation. The chitin content also increased with age, indicating an enhanced exoskeletal integrity and increased body length, particularly in larvae fed higher protein diets. Calcium and phosphorus levels peaked at the pupal stage, particularly in larvae of the CPmed and CPhigh groups, suggesting the role of these minerals in cuticle formation and structural development if the diet is optimised. This aligns with previous findings that nutrient composition in BSF varies according to developmental stage (Barragan-Fonseca et al., 2019). The observed nutrient changes reflect the altering metabolic demands across life stages, where early growth requires optimised protein and EAA availability. In contrast, later stages prioritise fat and chitin deposition for successful metamorphosis and reproduction. Understanding these stage-dependent variations is essential for optimising dietary formulations to enhance BSF growth, nutrient efficiency, and biomass production across the life cycle.

Dietary effects on insect nutrient composition

In addition to biological age (life stages), nutrition plays a critical role in determining the nutrient composition of H. illucens. Our study demonstrates that dietary protein content of 15% (CPmed) with a gross energy of 18.5 ± 0.3 MJ/kg DM was optimal for maximising larval growth, larval biomass gain, and adult size. In contrast, BSF fed a low-protein diet (10%) exhibited reduced live mass across the life cycle, lower protein content, and increased fat storage, indicating a metabolic trade-off favouring energy storage rather than growth. Excessive dietary protein concentrations (> 20%) reduced substrate intake, shown by lower substrate reduction in the CPhigh group compared to CPmed and CPlow. This may be due to decreased palatability or higher metabolic costs from excreting excess nitrogen (Nguyen et al., 2013).

The amount and the composition of the fatty acids of insect fat are highly variable and affected by both life stage and diet (Oonincx and Finke, 2025). As described by Oonincx and Finke (2025), the extent to which body fat content and fatty acid composition in insects can be altered depends strongly on the species. An increase in body fat may dilute the relative concentrations of other nutrients, such as protein and moisture, as previously shown for house fly larvae (Pearincott, 1960).

Oonincx and Finke (2025) reported that the calcium concentration in black soldier fly prepupae varied widely, ranging from 1 to 66 g/kg DM across three studies using nine different diets (Proc et al., 2020, Wang et al., 2020, Spranghers et al., 2016). While iron, zinc, and manganese levels fluctuated, phosphorus and potassium concentrations remained more stable. In our study, calcium and phosphorus levels peaked at the pupal stage and were higher in CPmed and CPhigh larvae than in CPlow larvae. These variations may be due to the mineralised exoskeleton of BSF, as CPmed BSF were larger and heavier than CPlow BSF.

Consequences of different *Hermetia illucens* nutrient compositions as a feed ingredient

This study demonstrated that both the developmental stage and the dietary composition significantly influence biomass production and nutrient composition in BSF larvae. The heterogeneity in the nutrient composition of larval biomass also affects the quality of the insect-derived products, e.g., insect meal as a source of protein, when used as an animal feed ingredient (Rossi et al., 2025, Dörper et al., 2025, Veldkamp and Vernooij, 2025). For instance, larvae have lower fat and chitin contents than prepupae, influencing digestibility in poultry, pigs, aquaculture species, and pets. This, in turn, affects animal performance, health and well-being (Rossi et al., 2025). For example, broilers fed with BSF meal composed of larvae achieved higher weight gain and feed conversion efficiency compared to those fed with meal composed of BSF prepupae despite both diets being iso-nitrogenous and iso-caloric (Dörper et al., 2025). Protein quality is also determined by digestibility and, hence, amino acid availability (Oonincx and Finke, 2025). Insect-based protein sources generally provide amino acids that are well-digestible for poultry, often matching or even surpassing the bioavailability of those from conventional feed ingredients like soybean or fish meal (De Marco et al., 2015). A notable exception is protein derived from black soldier fly larvae, which has been shown to have reduced digestibility, particularly regarding sulfur-containing amino acids such as methionine and cystine (Schiavone et al., 2017). The mineral composition of Hermetia illucens larvae and prepupae varies substantially with age and dietary inputs (Liland et al., 2017, Wang et al., 2020, Spranghers et al., 2016). As some of the protein fraction is associated with the insect's mineral-rich exoskeleton, high mineral content may hinder protein utilisation. Optimising larval diets to reduce mineral accumulation could therefore enhance protein digestibility in poultry. Moreover, variations in amino acid profiles and chitin content among insect species may also impact gut health and immune function in animals such as pigs, poultry, and fish (Rossi et al., 2025, Gasco et al., 2023). This is further discussed by Oonincx and Finke (2025), who emphasise the importance of larval diet-specific adjustments for improving insect meal digestibility and overall nutritional value.

Conclusion

This study demonstrates that both the nutrition of Hermetia illucens larvae and their life stage at separation are crucial for optimising their value as a feed source. Diets with optimised protein levels (15% CP in DM), at an adequate dietary energy content (18.5 MJ/kg in DM), result in increased biomass growth, faster development, larger adult size, and optimal protein and amino acid deposition. While fat deposition is reduced compared to the low-protein-fed larvae (10% CP in DM), the correct timing of separation, particularly at the prepupal stage, is also important. Higher dietary protein levels (20% CP in DM) did not improve performance. These findings highlight the significance of standardised feeding protocols and consistent harvesting times to optimise the nutritional profile of BSF.

Ethics approval

Not applicable.

Data and model availability statement

None of the data were deposited in an official repository. Infor mation can be made available from the authors upon request.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

The authors declare no conflictsofinterest.

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6 General discussion

The present study was designed to investigate the influence of substrate composition and dietary protein-to-energy ratio on the growth performance, nutrient utilisation, body composition, and overall efficiency of black soldier fly larvae (BSFL, *Hermetia illucens*) throughout their life stage development. The overarching aim was to identify nutritional parameters that optimize larval biomass yield and nutrient efficiency, thereby contributing to the development of sustainable insect-based feed production systems.

Animals, including insects, require nutrients and energy to support both maintenance and production processes. While maintenance refers to the preservation of basal physiological functions, production encompasses growth, reproduction, and tissue accretion (Cheeke and Dierenfeld, 2010). Insects share many fundamental nutritional requirements with vertebrates, including essential amino acids as well as sterols, minerals, and B-complex vitamins (Tomberlin et al., 2023). These nutrients must be supplied in bioavailable forms, which can be absorbed and metabolically utilized by the organism. Consequently, the nutritional value of a larval feed substrate is not solely determined by its chemical composition, but also by its digestibility, physical properties (e.g., particle size, water-holding capacity), and the presence of anti-nutritional factors.

Within circular bioeconomy strategies, black soldier fly larvae (BSFL) are increasingly framed as a scalable conversion platform for transforming low-value side streams into protein- and lipid-rich biomass (van Huis et al., 2013; Diener et al., 2009). A persistent bottleneck for industrial deployment, however, is performance variability when diets are selected or formulated primarily from crude nutrient concentrations; substantial differences across feedstocks have repeatedly been reported even when nominal proximate composition appears comparable (Gold et al., 2020a; Barragán-Fonseca et al., 2018). The thesis therefore builds on the premise that nutrient supply must be described more precisely at the level of utilisable nutrients per ingredient and that a utilisation-centred approach can improve predictability and efficiency.

To address this objective, three complementary experimental approaches were combined to balance industrial realism and mechanistic interpretability. Publication I (Chapter 3) screened seven food-industry by-products under commercial high-density rearing with repeated harvests, quantifying growth, survival, feed conversion, nutrient retention and frass composition across time. Publication II (Chapter 4) applied isoenergetic diets with graded protein levels to quantify retention efficiency of gross energy, nitrogen and amino acids, reporting the nitrogen-to-gross energy ratio (N:GE) as an integrative descriptor of protein supply relative to energy supply. Publication III (Chapter 5) extended the protein-gradient concept across life stages, reflecting that development alters nutrient composition and that both diet and separation timing affect conversion efficiency and feed value.

This chapter integrates the three studies in relation to the thesis hypotheses—(i) substrate type influences growth performance, nutrient utilisation efficiency and body composition; (ii) dietary protein concentration affects nutrient utilisation efficiency; and (iii) dietary protein concentration influences growth performance, developmental dynamics and nutrient composition across life stages—while emphasising mechanisms and implications rather than re-stating the results section.

6.1 Integration of key results in the scientific context

Publication I demonstrates that side streams differ fundamentally in their capacity to support stable, efficient BSFL production under commercial conditions. Under high-density rearing with repeated harvests, substrate type significantly affected growth, survival and feed conversion, with substrate-by-time interactions. Wheat bran produced the highest survival and biomass gain, whereas carrot scraps and grape pomace resulted in the lowest values. Such ranking effects align with evidence that BSFL outcomes are strongly feedstock-dependent and shaped by matrix properties and nutrient accessibility beyond proximate composition (Barragán-Fonseca et al., 2018; Spranghers et al., 2017; Gold et al., 2020a). Nitrogen retention also differed across substrates, underscoring that substrate choice can shift nutrient partitioning in addition to yield.

The substrate set was chosen for European availability and practical relevance. Carrot pomace was described as a fibrous juice-production by-product; grape pomace as a heterogeneous residue consisting of skins, seeds and stems; bakery by-products (biscuit meal and bread crumbs) as energy-dense residues from overproduction and returns; and wheat bran as a widely available milling by-product rich in fibre, protein and minerals. The observed ranking supports the view that substrate “composition” extends to matrix properties such as moisture behaviour, fibre structure, polyphenolic fractions and substrate ageing, which together shape nutrient accessibility and feeding dynamics under high density (Gold et al., 2020a; Barragán-Fonseca et al., 2018). Comparable constraints have been reported for grape pomace and similar residues, which often require blending or processing to achieve consistent performance (Ribeiro et al., 2022).

A central contribution of Publication I is the explicit inclusion of nutrient retention and frass composition. Energy-related outcomes favoured bakery by-products relative to carrot scraps and grape pomace, whereas nitrogen retention patterns differed and favoured wheat bran. This decoupling is important for systems positioned as protein solutions and mirrors biowaste treatment studies emphasising that evaluation must track both biomass yield and carbon/nitrogen conversion (Gold et al., 2020a; Pang et al., 2020).

Frass composition closely reflected substrate nutrient composition—particularly nitrogen and energy—and harvest timing influenced both larval and frass composition. Therefore, circularity depends on nutrient partitioning between biomass and residual streams, not only on larval yield. Reviews and fertilisation studies emphasise that frass performance hinges on nitrogen speciation and mineralisation dynamics and that these properties vary strongly with substrate and processing (Lopes et al., 2022; Basri et al., 2022; Gärttling and Schulz, 2020). Moreover, nitrogen fate cannot be inferred from retention alone because part of the non-retained fraction may be transformed into gaseous losses during rearing or subsequent handling (Pang et al., 2020; Ermolaev et al., 2019).

Publication II isolates dietary protein effects under isoenergetic supply and clarifies a mechanistic driver of utilisation. Diets ranged from CP10 (102 g/kg crude protein) to CP20 (202 g/kg crude protein), with similar gross energy; N:GE increased from 0.89 (CP10) to 1.72 (CP20), with intermediate values at CP14 (1.26) and CP16 (1.43). Total amino acids increased from 81.8 g/kg DM in CP10 to 173.8 g/kg DM in CP20. This framing is consistent with macronutrient-balance studies indicating that BSFL responses are governed by protein–energy relations rather than crude protein alone (Cammack and Tomberlin, 2017; Chia et al., 2020; Bellezza Oddon et al., 2022a).

Critically, nutrient utilisation efficiency was non-linear. Nitrogen retention peaked at 61.6% in CP14-fed larvae but fell to 39.1% at CP20; total amino acid retention declined from 58.0% at CP14 to 33.9% at CP20. Retention of essential amino acids (including lysine, methionine, threonine, tryptophan and valine) was higher in larvae fed CP14 than CP20, consistent with reports that conversion efficiency can deteriorate under protein-biased or otherwise unbalanced diets (Cammack and Tomberlin, 2017; Bellezza Oddon et al., 2022).

These data substantiate a central implication of the thesis: oversupplying protein relative to energy can reduce the fraction of dietary nitrogen and amino acids retained in harvestable biomass, despite higher nutrient provision. This interpretation is congruent with broader insect nutrition literature, where surplus amino acids are increasingly catabolised and excreted when energetic or anabolic constraints limit deposition (Tomberlin et al., 2023).

A parsimonious interpretation is bounded anabolic deposition under metabolic balance constraints. Under isoenergetic supply, raising protein increases nitrogen exposure without increasing energy supply; beyond an intermediate range, surplus amino acids are increasingly diverted away from deposition, lowering retention efficiency. In waste-treatment contexts, diverted nitrogen can accumulate in residual streams and, depending on management, contribute to ammonia and other gaseous losses (Pang et al., 2020; Ermolaev et al., 2019).

Publication III explicitly frames optimisation as linked to development and separation timing, emphasising that nutrient composition changes across development and that antinutritional factors such as chitin and ash increase with age and may impair nutrient absorption and feed value. These developmental shifts are documented for *H. illucens* and motivate stage-specific optimisation and processing decisions (Caligiani et al., 2018).

6.2 Evaluation of the dissertation hypotheses

Hypothesis (i) — that variations in substrate type influence the growth performance, nutrient utilisation efficiency, and body composition of *Hermetia illucens* larvae — is supported by Publication I: substrate type significantly affected growth, survival and feed conversion with substrate-by-time interactions; wheat bran yielded the highest survival and biomass gain, while carrot scraps and grape pomace performed worst. Nitrogen retention was greatest under wheat bran and lowest under wheat pulp and rapeseed cake, demonstrating substrate effects on utilisation efficiency rather than growth alone. Frass outcomes further confirm that substrate differences extend to nutrient partitioning and co-product profiles, thereby affecting larval body composition and nutrient allocation patterns.

Hypothesis (ii) — that dietary protein concentration affects the nutrient utilisation efficiency of *Hermetia illucens* larvae — is supported by Publication II, with the important refinement that the relationship is non-linear. Under isoenergetic conditions, nitrogen and total amino acid retention were highest at CP14 and lowest at CP20, and essential amino acid retention followed the same direction. The hypothesis is therefore confirmed in the sense that protein level affects utilisation efficiency, while the thesis provides the more informative conclusion that protein oversupply can reduce efficiency.

Hypothesis (iii) — that dietary protein concentration enhances growth performance, developmental dynamics, and nutrient composition of *Hermetia illucens* across its life stages — is addressed by Publication III through its life-stage design and explicit motivation that development alters composition and feed value. By linking dietary protein to performance, developmental timing, and nutrient composition across stages, the study supports the premise that dietary protein influences developmental dynamics and stage-specific nutrient outcomes. At thesis level, this reinforces that optimisation must be endpoint-defined and interpreted relative to a specified harvest stage.

6.3 Biological, theoretical and methodological interpretation

The most mechanistically informative result of the thesis is the identification of an intermediate protein-to-energy range that maximises nitrogen and amino acid retention under isoenergetic supply. The CP14 diet (N:GE 1.26) achieved the highest nitrogen retention (61.6%) and total amino acid retention (58.0%), whereas CP20 (N:GE 1.72) showed markedly lower retention (39.1% nitrogen; 33.9% total amino acids). The decline at high protein supply, despite higher dietary amino acid concentrations, may indicates that anabolic incorporation is constrained and that increasing fractions of amino acids are diverted away from deposition pathways when protein-to-energy exposure becomes excessive.

Two aspects strengthen this interpretation for applied formulation. First, the efficiency loss at CP20 is not marginal but substantial, indicating that beyond the utilisation optimum a considerable share of dietary nitrogen does not contribute to harvestable amino acids. Second, the decline affects multiple essential amino acids (e.g., lysine, methionine, threonine, tryptophan and valine), suggesting that the phenomenon reflects a system-level metabolic constraint rather than a single limiting amino acid effect.

In practice, this implies that the nutritional value of the resulting biomass, particularly its essential amino acid profile, may be better protected by avoiding protein oversupply than by maximising crude protein inclusion.

A further theoretical implication is the utility of N:GE as a formulation-relevant descriptor. Because isoenergetic diets disentangle protein supply from energy supply, the observed optimum supports interpreting protein effects through protein-to-energy balance rather than through crude protein percentage alone. This is particularly relevant for circular feeding strategies using heterogeneous substrates where gross energy and protein density may co-vary unpredictably.

The life-stage perspective adds a further layer: even if a diet approaches a utilisation optimum at one stage, developmental processes shift composition and thereby the relevance of traits such as chitin and ash for downstream digestibility. Publication III emphasises that antinutritional factors increase with age and may impair nutrient absorption and feed value. This provides a mechanistic rationale for integrating harvest timing into optimisation logic, rather than treating diet as the sole lever. In particular, a strategy that slightly reduces maximal biomass but stabilises harvesting at a larval stage with lower chitin/ash may improve functional feed value, depending on the target species and formulation constraints.

Methodologically, this implies that diet trials restricted to a single harvest point risk conflating diet effects with stage effects unless harvesting is standardised and stage definition is explicit. Publication III therefore supports harmonised separation protocols both for research comparability and for industrial product consistency.

Interpreting Publications I and II together supports a two-layer model of variability. First, substrate matrix properties and rearing dynamics determine effective nutrient availability under commercial conditions (Gold et al., 2020a; Barragán-Fonseca et al., 2018). Second, physiological utilisation constraints impose an efficiency optimum for protein-to-energy balance, explaining why high protein provision does not automatically convert into high retained amino acid mass (Cammack and Tomberlin, 2017; Chia et al., 2020).

6.4 Limitations and consequences for interpretation

Several limitations define the scope of inference. Side streams are intrinsically variable across suppliers and processing conditions; therefore, the substrate rankings in Publication I should be interpreted as robust outcomes for the tested materials under the specific experimental conditions, rather than as immutable properties of each by-product category (Gold et al., 2020a; Barragán-Fonseca et al., 2018). Commercial rearing also entails complex, interacting influences of substrate ageing, microbial dynamics, and physical matrix changes, which can obscure the attribution of observed effects to specific nutrient fractions.

Across the three studies, inference is additionally constrained by the limited number of substrate batches and by the fact that side streams were sampled from specific suppliers and processing contexts. This highlights the need for multi-batch validation and for screening protocols that explicitly quantify within-category variability, as also emphasised in systematic and review work on substrate effects (Hopkins et al., 2021; Gold et al., 2020a).

Retention metrics quantify incorporation into biomass but do not specify the chemical fate of the non-retained fraction. Nitrogen speciation in frass and potential emissions during rearing and storage were not measured, constraining direct conclusions about environmental performance. Empirical studies show that BSFL treatment can involve measurable NH_3 and N_2O emissions that depend on substrate properties and process conditions, motivating paired retention and emission monitoring (Pang et al., 2020; Ermolaev et al., 2019).

Finally, endpoint definition remains crucial: Publication III emphasises that development alters composition and feed value; thus, numerical optima from isoenergetic diets should be treated as mechanistic reference points that require alignment with harvest stage and product specification before transfer to industrial substrates.

6.5 Practical and applied relevance for circular feeding strategies

Publication I provides a direct operational message: substrate selection decisively affects production stability and efficiency under high-density, scalable rearing. Wheat bran supported superior survival, biomass gain and conversion efficiency, whereas carrot scraps and grape pomace resulted in consistently weak performance. This supports structured substrate qualification rather than opportunistic feeding based on availability alone (Gold et al., 2020a; Hopkins et al., 2021).

Publication II adds a formulation principle with direct economic relevance: protein oversupply can reduce nitrogen and amino acid retention, lowering the return on protein-rich ingredients that often dominate diet costs. This aligns with diet-manipulation work indicating that BSFL conversion is strongly shaped by macronutrient balance (Cammack and Tomberlin, 2017; Chia et al., 2020). Applied to side streams, it supports targeting a protein-to-energy range and using retention-based indicators rather than crude protein percentage alone.

Publication III strengthens the applied conclusion that diet optimisation must be integrated with separation timing and standardised harvesting protocols to stabilise product composition and digestibility-relevant traits. Together, the three studies support an applied strategy in which substrates are selected and managed to ensure stable effective nutrient availability, dietary protein supply is aligned with energy supply to remain within a utilisation-optimal range, and harvesting targets a defined developmental endpoint and product profile.

6.6 Outlook for future research

Future research should translate retention-based findings into predictive diet formulation frameworks capable of operating under industrial heterogeneity. From a nutritional physiology perspective, this requires integrating the dietary protein-to-energy ratio (expressed relative to metabolisable energy) with physicochemical substrate characteristics that determine nutrient availability, alongside process-related variables such as larval density, substrate ageing, moisture content and microbial dynamics (Gold et al., 2020a; Hopkins et al., 2021). Mechanistic models should move beyond crude protein concentration towards digestible amino acid supply, stage-specific amino acid requirements, and metabolic utilisation efficiency under commercially relevant conditions. Importantly, formulation approaches must distinguish between dietary protein density (composition of the substrate), protein intake (resulting from feeding behaviour and intake regulation), and cumulative protein-to-energy exposure across developmental time.

A second priority concerns resolving nitrogen fate within BSF production systems. Robust circularity and environmental assessments require detailed nitrogen speciation in frass, together with quantification of gaseous emissions and leaching-relevant losses during rearing and storage. Fertiliser value depends on nitrogen form rather than total nitrogen concentration alone, whereas environmental impacts are determined by volatilisation losses and nitrification–denitrification pathways (Lopes et al., 2022; Basri et al., 2022; Pang et al., 2020; Ermolaev et al., 2019). A physiologically grounded nitrogen balance framework linking nitrogen intake, retention efficiency, and partitioning into body protein, frass nitrogen fractions and gaseous emissions will therefore be essential.

A third research priority is the development of rapid and robust substrate qualification protocols suited to industrial decision-making. Given the expected within-category variability of organic side-streams, rapid compositional indicators, near-infrared calibration models, or short-term performance bioassays could enable screening of incoming batches prior to large-scale deployment. The time-dependent responses observed in Publication I indicate that substrate qualification should account for developmental dynamics rather than relying exclusively on single time-point performance metrics.

From a developmental nutrition perspective, dynamic feeding strategies warrant investigation. Stage-specific adjustment of digestible amino acid supply, while maintaining the dietary protein-to-energy ratio within the established utilisation optimum at the intended harvest stage, may enhance early protein deposition without increasing cumulative nitrogen losses. Such strategies should be integrated with standardised harvest timing to stabilise nutrient composition and functional quality of the resulting larval biomass.

Further research should refine stage-specific nutrient requirement models grounded in intake regulation, retention efficiency and metabolic partitioning. Harmonised methodologies for assessing digestible amino acid availability, protein quality indices and functional lipid characteristics will be essential to ensure reproducibility and comparability across studies. Interdisciplinary integration of insect physiology, nutritional biochemistry and animal production science will be required to translate larval performance data into predictive feeding systems for livestock and aquaculture.

The successful incorporation of insect-derived ingredients into animal diets ultimately depends on a mechanistic understanding of how developmental stage, dietary protein-to-energy ratio, amino acid profile and processing influence nutrient availability and biological value. Optimising these parameters through targeted rearing and harvest strategies will enhance both nutritional efficacy and nitrogen-use efficiency. In this context, *Hermetia illucens* represents not merely a bioconverter of organic side-streams, but a controllable nutritional production system within circular, resource-efficient feed chains.

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8 Summary

The standardised rearing of black soldier fly larvae (BSFL, *Hermetia illucens*) is gaining increasing relevance within sustainable livestock production systems. These insects efficiently convert organic by-products into nutrient-dense biomass suitable for incorporation into feed for farmed and companion animals. Targeted optimisation of larval nutrition is essential to enhance nutrient utilisation efficiency and to improve the nutritional value of the resulting biomass, with dietary protein concentration representing a key regulatory factor. Despite the growing industrial importance of BSFL production, substantial knowledge gaps remain regarding optimal dietary protein levels, particularly in relation to specific developmental stages.

Across three interrelated studies, a consistent pattern emerged: dietary protein concentration not only modulated growth performance but also influenced nutrient utilisation, body composition, nitrogen retention, and the partitioning of protein and lipid deposition during ontogeny. BSFL demonstrated the capacity to valorise a broad spectrum of organic sidestreams; however, performance was strongly dependent on substrate type and nutrient composition. In Publication I, distinct substrates exerted significant effects on growth, survival, feed conversion efficiency, and nitrogen retention. Wheat bran consistently supported superior performance, whereas carrot and grape residues resulted in reduced growth rates and lower utilisation efficiencies. The nutrient profile of the frass reflected the nitrogen and energy characteristics of the respective substrates, underscoring the close interrelationship between substrate composition, larval metabolism, and residual nutrient dynamics.

A central component of the research involved the evaluation of graded crude protein concentrations in isoenergetic diets (18.5 MJ kg dry matter). In Publication II, diets containing 10, 14, 16, and 20% crude protein were assessed. A dietary concentration of approximately 14% proved physiologically optimal. Both lower and higher protein levels impaired nutrient utilisation efficiency: protein deficiency constrained protein biosynthesis and reduced nitrogen and amino acid deposition, whereas excess protein did not yield proportional performance gains but was associated with increased mineral deposition, particularly calcium.

Publication III extended this analysis across the complete life cycle (10, 15, and 20% crude protein at 18.5 MJ kg dry matter). From approximately day 10 onwards, larvae receiving protein-deficient diets exhibited reduced body mass relative to those fed moderate or higher protein levels. Moderate protein supply accelerated progression to the prepupal stage and

resulted in elevated dry matter, protein, and amino acid concentrations at this stage. Larvae reared on low-protein diets exhibited compensatory lipid deposition during the prepupal phase.

Collectively, the findings demonstrate that increasing dietary protein concentration does not linearly enhance performance. An optimal range of 14–15% crude protein at 18.5 MJ kg dry matter maximised nitrogen and amino acid utilisation. Protein levels exceeding this range increased nitrogen excretion without further biomass accretion. Moreover, stage-specific patterns were evident: early instars prioritised protein and essential amino acid deposition to support rapid tissue growth, whereas advancing development was characterised by increased lipid deposition and structural investment, including chitin formation. Dietary protein supply modulated these physiological processes and determined the relative allocation of protein and lipid within larval biomass. Insufficient protein intake induced a compensatory shift towards lipid deposition, whereas excessive protein supply resulted in elevated nitrogen losses due to increased excretion. The positive association between larval and adult body size suggests that larval nutrition may exert carry-over effects on subsequent life stages, including reproductive performance. Thus, both larval diet and developmental stage critically determine larval nutrient composition, with direct implications for feed value and resource-use efficiency.

Future research should establish standardised feeding protocols and clearly defined harvest points to ensure consistent nutrient composition in insect-derived products and to optimise production efficiency within BSF rearing systems.

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9 Zusammenfassung

Die standardisierte Aufzucht von Larven der Schwarzen Soldatenfliege (BSFL, *Hermetia illucens*) wird im Rahmen einer nachhaltigen Tierproduktion immer wichtiger. Diese Insekten können organische Nebenprodukte effizient in nährstoffreiche Biomasse umwandeln, die als Futtermittel für Nutz- und Heimtiere genutzt wird. Eine gezielte Optimierung der Insektenernährung ist entscheidend, um die Nährstoffverwertung zu verbessern und den Futterwert der Larvenbiomasse zu steigern. Dabei spielt insbesondere der Proteingehalt der Nahrung eine zentrale Rolle. Trotz der zunehmenden Bedeutung der BSFL-Produktion gibt es jedoch weiterhin erhebliche Wissenslücken hinsichtlich der optimalen Proteingehalte in der Nahrung, insbesondere in Abhängigkeit vom jeweiligen Entwicklungsstadium der Insekten.

Die Ergebnisse über drei zusammenhängende Studien zeigen ein konsistentes Muster: Der Proteingehalt der Nahrung moduliert nicht nur die Wachstumsrate, sondern beeinflusst auch die Nährstoffverwertung, die Körperzusammensetzung, die Stickstoffretention sowie das Verhältnis zwischen Protein- und Fettansatz während der Entwicklung der Insekten. Die Larven der Schwarzen Soldatenfliege können eine breite Palette organischer Nebenströme in wertvolle Biomasse umwandeln, wobei ihre Leistungsfähigkeit stark vom Substrattyp und deren Nährstoffzusammensetzung abhängt. In Veröffentlichung I wurde gezeigt, dass unterschiedliche Substrate signifikante Effekte auf Wachstum, Überlebensrate, Futterverwertung und Stickstoffretention haben. So führte Weizenkleie konsistent zu den höchsten Leistungen, während Karotten- oder Traubenreste geringere Wachstumsraten und eine niedrigere Effizienz verursachten. Das Nährstoffprofil des Frasses spiegelte dabei die Stickstoff- und Energieeigenschaften der Substrate wider, was die enge Verbindung zwischen Substratzusammensetzung, Larvenmetabolismus und Reststoffdynamik verdeutlicht.

Ein zentraler Bestandteil der Forschung war die Bewertung abgestufter Proteingehalte in isoenergetischen Diäten. In Veröffentlichung II wurden Rohproteingehalte von 10, 14, 16 und 20 % bei 18,5 MJ kg Trockensubstanz an BSFL gefüttert. Ein Rohproteingehalt von etwa 14 % erwies sich als physiologisch optimal. Sowohl niedrigere als auch höhere Proteingehalte führten zu Effizienzeinbußen. Während ein Proteinmangel (10 %) die Proteinbiosynthese der BSFL limitierte und sich negativ auf Wachstum sowie Stickstoff- und Aminosäureansatz auswirkte, resultierte ein Überschuss (20 %) nicht in zusätzlichen Leistungssteigerungen. Stattdessen wurde eine erhöhte Mineraleinlagerung (Calcium) beobachtet.

Veröffentlichung III erweiterte diese Analyse auf den gesamten Insektenlebenszyklus (10, 15 und 20 % Rohprotein bei 18,5 MJ kg). Ab etwa Tag 10 wiesen Larven mit proteinarmen Diäten geringere Körpermassen auf als solche, die mit moderaten oder höheren Proteingehalten gefüttert wurden. Larven mit moderaten Proteingehalten erreichten schneller das Präpuppenstadium und zeigten in dieser Phase höhere Trockenmasse-, Protein- und Aminosäurekonzentrationen. Larven der proteinarmen Diäten kompensierten durch erhöhten Fettansatz im Präpuppenstadium.

Die Ergebnisse verdeutlichen, dass ein höherer Proteingehalt in der Nahrung nicht automatisch zu gesteigerter Leistungsfähigkeit führt. Optimal erscheint ein Bereich von 14–15 % Rohprotein bei 18,5 MJ kg Trockensubstanz, der die Stickstoff- und Aminosäurenverwertung maximiert. Überschreitet der diätetische Proteingehalt diesen Bereich, kommt es zu einer erhöhten Stickstoffausscheidung ohne zusätzlichen Biomassezuwachs. Die Studien zeigen zudem entwicklungsstadiumspezifische Muster: Juvenile Larven priorisieren Protein- und essentielle Aminosäureansatz zur Unterstützung schnellen Gewebewachstums, während mit zunehmendem Alter der Fettansatz steigt und strukturelle Komponenten wie Chitin aufgebaut werden. Die diätetische Proteinzufuhr moduliert diese physiologischen Prozesse und beeinflusst die relativen Anteile von Protein- und Fettansatz im Larvenkörper. Eine unzureichende Proteinzufuhr führt zu einer kompensatorischen Verschiebung des Nährstoffansatzes zugunsten eines erhöhten Fettansatzes. Demgegenüber resultiert eine übermäßige Proteinzufuhr nicht in einer weiteren Steigerung des Proteinansatzes, sondern in erhöhten Stickstoffverlusten infolge gesteigerter Exkretion. Die positive Korrelation zwischen Larven- und Adultkörpergröße deutet darauf hin, dass die Ernährung während der Larvenentwicklung auch spätere Lebensstadien, einschließlich reproduktiver Leistung, beeinflusst. Somit beeinflussen sowohl die larvale Ernährung als auch das Entwicklungsstadium der BSF maßgeblich die Nährstoffzusammensetzung der Larven. Dies wirkt sich wiederum auf ihre Eignung als Futtermittelkomponente sowie auf die Ressourceneffizienz der Nährstoffverwertung aus.

Zukünftige Forschung sollte standardisierte Fütterungsprotokolle und definierte Erntezeitpunkte etablieren, um eine konsistente Nährstoffzusammensetzung in insektenbasierten Produkten sicherzustellen und die Produktionseffizienz in BSF-Systemen zu optimieren.

List of Publications

Schneider, L., Stoehr, N., Kisinga, B., Cord-Landwehr, S., Weiss, C. K., Schulte-Geldermann, E., Moerschbacher, B. M., Eder, K., Jha, R., and Dusel, G. 2025. Effects of various by-products of the food industry on performance and energy and nitrogen retention in black soldier fly larvae. *Submitted to Insects as Food and Feed.*

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Schneider L., Stoehr N., Dusel G. 2025. Influence of dietary fat sources on performance, body composition, and fatty acid profiles in *Hermetia illucens* larvae. Proc. Soc. Nutr. Physiol. 34, 85.

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Erklärung

Ich erkläre: „Ich habe die vorgelegte Dissertation selbstständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nicht veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ niedergelegt sind, eingehalten.“

Gießen, 21.02.2026 Laura Schneider

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