



CLEAN AND GREEN BIOCONVERSION – A COMPREHENSIVE REVIEW ON BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) LARVAE FOR CONVERTING ORGANIC WASTES TO QUALITY PRODUCTS

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Abstract

Food security remains a pressing global concern, exacerbated by population growth, diminishing agricultural lands, and climate uncertainties. As the demand for high-quality protein sources like eggs, meat, and milk escalates, conventional feed ingredients face challenges in meeting the burgeoning needs of livestock production. The projected increase in poultry and pig consumption further strains the availability of protein-rich feed sources, necessitating sustainable alternatives. Insects, notably black soldier fly larvae (BSFL), offer numerous advantages, including efficiently converting organic substrates into high-quality protein, fat, minerals, and vitamins. Their rapid reproduction, minimal environmental footprint, and ability to thrive on various organic materials make them an attractive protein source. However, consumer acceptance remains a hurdle, hindering their direct consumption despite their nutritional value. Incorporating BSFL into animal diets, especially poultry and swine, demonstrates promising results regarding growth and production. This review comprehensively overviews BSFL production systems, processing techniques, and nutritional profiles. Various factors influencing BSFL growth and feed quality are discussed, highlighting the importance of optimizing breeding systems and feed formulations. Processing methods are elucidated to ensure the safety and quality of BSFL-based products. Nutritional analysis reveals BSFL as a rich source of essential amino acids, fatty acids, and minerals, making them suitable replacements for soybean meal and fish meal. Despite the economic and environmental benefits of BSFL utilization, challenges persist, including regulatory issues, consumer perceptions, and production scalability. Standardized production protocols and legislative frameworks are needed to facilitate the widespread adoption of BSFL in animal feed industries. In conclusion, integrating BSFL into animal diets presents a promising solution to address protein shortages in livestock production while promoting sustainable resource utilization.

Key words: bioconversion, black soldier fly, growth performance, immunology, poultry, processing, rearing

Food security is alarming as more than one billion people face insufficient dietary energy sources worldwide (Wudil et al., 2022). It is essential to fulfill the demand for eggs, meat, and milk for human consumption associated with challenges for water shortages (Davis and White, 2020) along with the decrease in agricultural lands due to unpredicted weather and climate changes, as the population of the world is increasing day by day. The world population is predicted to be more than 9.7 billion by 2050 (Gu et al., 2021), and consumption of poultry and pig is anticipated to increase by 173% and 105%, respectively (McLeod, 2011; Tiengtam et al., 2017). This massive increase in pig and poultry production will significantly create hurdles in providing feed ingredients containing protein, which are likely to be more than 1300 million tons of dry matter (Paengkoum and Paengkoum, 2010). The primary protein sources for ruminants and monogastric animals are some oilseed cakes and

soy grains (such as soybean meal, cottonseed meal, and rapeseed meal) (Kaewwongsa et al., 2011). Protein is an expensive and limited component of feed formulations and traditional sources; however, costs have increased dramatically due to yield-related issues and competition between people and animals (Chen et al., 2010; Van Huis and Oonincx, 2017). The future will encourage animal production systems to discover sustainable alternative protein sources to fulfill the need for high-density animal feed. In this situation, while there is less competition between human and animal food chains for plant-based protein sources, economic and environmental considerations must be taken into account (Allegratti et al., 2018; Dicke, 2018; Purba et al., 2020 b). To increase the growth and milk production capabilities of ruminants, urea could be used up to certain levels, in their diets as a source of non-protein nitrogen (Wanapat et al., 2000; Paengkoum et al., 2006 a, b; Vorlaphim et al., 2021). However, in

monogastric species, non-protein nitrogen sources cannot be used because elevated concentration of ammonia in gastrointestinal tract damages the gastrointestinal mucosa, affecting nutrition absorption, energy efficiency, and growth performance (Patra and Aschenbach, 2018). Therefore, a suitable alternative protein resource could be insects' protein.

Black soldier fly larvae (*Hermetia illucens*), mealworm larvae (*Tenebrio molitor* L.), and cricket (Orthoptera: Gryllidae) are prominent insects in emerging research realms as they can be used as protein raw materials as well as substitute or replacement ingredients for poultry and swine feed (Oonincx and De Boer, 2012; Schiavone et al., 2017 b; Gasco et al., 2019). The survival rate and nutritional value of BSFL are not affected significantly by dietary manipulation (Oonincx et al., 2015), with higher feed conversion rate, short reproductive cycle, and extensive content of fat, protein, minerals, and vitamins as its economic traits (Spranghers et al., 2017 b). In addition, they are mediated as more ecofriendly than conventional protein sources as they can be extensively cultivated in organic streams with a minimum budget (Meneguz et al., 2018 b; Tian et al., 2020). According to numerous researchers, BSFL can be used as human food and animal feed, which could eventually help address the world's food shortage. Consumers, however, prefer the usage of BSFL in feed instead of human consumption because there is some psychological resistance to consuming insects (Bukkens, 1997; Nyakeri et al., 2017; Tabata et al., 2017). BSFL-fortified diet for pigs and poultry ensured the digestibility and growth performance when compared to other protein sources (Veldkamp and Bosch, 2015). Similarly, broilers' growth performance, immunity, and intestinal health were not affected by replacing 50–100% soybean oil with whole-fat BSFL (Schiavone et al., 2018). As with poultry manure, BSFL may also effectively reduce its nutrient content, which is advantageous for enhancing farm hygiene (Zhou et al., 2013; Nguyen et al., 2015), lowering pest populations, and lowering runoff nutrient pollution (Mallin and Cahoon, 2003). The standard protein sources of soybean meal and fish meal could be replaced with BSFL. This review will extensively provide information about BSFL, its production system, processing, nutritional profile, and the significance of its use in poultry feed.

Methodology

A systematic research strategy was employed to identify relevant research for this review. Key scholarly databases (Google Scholar, Scopus, Web of Science, and PubMed) were comprehensively searched using a combination of keywords and search queries. Additionally, reference lists of prominent and recent publications were reviewed for relevant studies. Only English-language articles published before December 2023 were included. A dual-reviewer process ensured selection rigor, with disagreements resolved through discussion or a third reviewer. Selection criteria focused on study relevance,

methodological quality, and evidence strength. Data extraction from selected articles used a standardized format to capture key information on methods, results, and conclusions. This narrative synthesis approach facilitates the identification of trends, patterns, and knowledge gaps within the existing literature.

BSFL (*Hermetia illucens*)

The saprophytic insect BSFL mainly consumes organic wastes like agriculture byproducts, straws, plant leftovers, animal manure and trash, and food waste (Zheng et al., 2012; Lalander et al., 2013; Banks et al., 2014). The order Diptera has five genera in the subfamily Hermetiinae, one of which is BSFL (Woodley, 2011). *Hermetia illucens* is the species with the most significant geographic distribution, followed by *Patagiomysia*, *Chaetosargus*, *Notohermetia*, and *Chaetohermesia* (Singh and Kumari, 2019). It is a vast, slender, black species with brownish wings and appendages extending from the head, and it has three segments: the head, thorax, and belly (Üstüner et al., 2003). The abdomen has five segments with white dots on it. Male BSF is more significant than female; however, its end genitalia and wings are more diminutive. The female BSF wings are between 8 and 14.8 mm long, and the body length is 12 to 20 mm (Üstüner et al., 2003). Egg, larva, pupa, and adult are the phases of their life cycle, from which the most nutrient-dense phases are the larval and pupal ones, having roughly 18–33% fat and 32–53% protein (Chippindale et al., 1993; St-Hilaire et al., 2007; Yu et al., 2009). Adult worms are harmless to other species and do not prefer the human body or raw food. They do not have mouth, digestive system, or stinger. As a result, they are also ineffective as disease-transmission vectors (Sheppard et al., 2002). The ideal temperature range for BSFL production is between 25 to 30°C (James, 1935; Shumo et al., 2019 b). They are widely dispersed in temperate, subtropical, and tropical climates. They cannot survive cold climates, such as those in north-west Europe and areas with temperatures below 5°C (Spranghers et al., 2017 a).

In contrast to being a great source of protein for both animals and humans, BSFL can also help to reduce pollution by using organic waste (Erickson et al., 2004; Paengkoum and Paengkoum, 2010; Purba et al., 2020 b, c). During decomposing trash, BSFL transforms organic waste into lipids, chitin, vitamins, amino acids, peptides, and proteins, which help vitamins, amino acids, peptides, and proteins, which help control pests and perilous bacteria like *Salmonella* and *Escherichia coli*. These products are also utilized in chemical manufacturing and medicine for poultry and pig feeds (Erickson et al., 2004; Liu et al., 2008). Moreover, valuable amino acids, proteins, and fats can be produced from the conversion of more than fifty percent of poultry waste. It can be further used in animal feed as it is cost-effective for rearing BSFL. BSFL additionally contains a higher content of chitin and minerals that are highly resistant and have antioxidant effects (Wang et al., 2020).

BSFL production systems

BSFL is commonly utilized as an agent to process organic waste because it is simple to handle and very cheap to create and does not require a high-tech system (Diener et al., 2011 a; Nguyen et al., 2015; Kim et al., 2021). For the system to be effective and adaptable globally, methodological protocols for BSF processing should be established. While rearing BSF for breeding, organic waste should be adequately available for feeding. Shortage of feed availability may result in the mortality of flies in a short time. Additionally, the design should be flexible enough to respond to the socio-cultural circumstances of nearby populations.

Availability of organic materials

The polyphagous insect BSFL mainly feeds on all varieties of animal and plant-based organic matter (Nguyen et al., 2015; Bava et al., 2019). BSFL can convert municipal garbage into a useful product at a variable rate from 65.5 to 78.9%. Although municipal garbage harms BSFL growth, BSFL can remove dangerous elements from waste streams, such as zinc (Diener et al., 2011 a). However, rearing on vegetable wastes (Kinasih et al., 2018), fish offal (Pamintuan et al., 2019), and animal manure (Parodi et al., 2020) have demonstrated the adaptability of organic waste into BSFL with bio-conversion.

Chattels of biotic and abiotic factors

BSF could be quickly grown in a range of environments; however, relative humidity and temperature have a preeminent influence on its performance (Tomberlin et al., 2009; Holmes et al., 2012; Holmes et al., 2016). To attain outstanding results, the impact of the abiotic factor should be deemed even at the minor level for the breeding system. The growth of larvae, prepupae, pupae, and adult BSF are all influenced by temperature, which is the most significant abiotic factor. An optimal temperature of 30 degrees Celsius increased population growth due to factors such as the survival rate of immature life stages and temperature conditions (Chia et al., 2018). BSFL can survive at low temperatures, such as 19°C; however, 12°C to 16°C is required for hatching eggs. This data is useful for breeding programmers around the globe, especially in extensively temperature-varied regions (Holmes et al., 2016). Temperature and humidity should be monitored for rearing and ideal breeding of BSF (Holmes et al., 2012). A greater relative humidity would accelerate the success of egg hatching and flies' growth. Along with abiotic factors, larval density per breeding container impacted the quality of the nutrients in the larvae, particularly crude fat content, but not protein content. BSF can be cultivated in environments with various abiotic conditions; however, optimum abiotic and biotic conditions should be considered for commercial breeding (Barragan-Fonseca et al., 2018).

Starting with local BSF

One of the simplest ways to establish the colony is to capture adult BSF from the wild, whether breeding on

a small scale or at an individual level. However, specific techniques like attracting organic waste are required to entice adult BSF to appear and lay eggs. For instance, Ewusie et al. (2019) found that manure from pigs, sheep, and chickens is a tempting substrate for collecting BSF eggs; however, manure should be readily available in the venue. The findings demonstrated that BSF preferred pig manure as an egg-laying medium. However, Sripontan et al. (2017) demonstrated that BSF preferred fruit waste over other wastes, such as cattle waste, for laying eggs. These two studies demonstrate a BSF preference for organic waste with some regional variations. Female BSF selects media that can shield the eggs from predators and the damaging impacts of abiotic variables, including dryness and sunshine (Dortmans et al., 2017).

Establishment of BSFL breeding unit

Two breeding strategies are commonly used to meet community conditions: the artificial mass breeding system and the natural colonization structure. Breeding systems aim to minimize waste and produce income. The initial method should be used in homes or communities with restricted space based on local regulations and the availability of essential facilities. (Čičková et al., 2015; Lohri et al., 2017). The second approach, better controlled, is employed by a for-profit organization with the dual goals of increasing revenue and implementing a comprehensive organic waste disposal program.

The nutritional analysis of this system tried to provide affordable protein for fish and poultry (Nyakeri et al., 2017). A natural breeding system has been established in Kenya. The rearing strategy uses mashed maize grains, surplus fruit and vegetable scraps, fish, and local manures. It also confirmed the system's ability to produce sufficient larval biomass to meet the nutritional needs of both fish and poultry. In addition, it is demonstrated that a miniature breeding system using mobile tanks could generate adequate feed to breed tilapia at their best (Gougbedji et al., 2021). This is economically advantageous, particularly in middle-class societies, as a source of income.

In contrast, artificial systems employ cutting-edge management techniques and machinery to treat enormous quantities of organic waste and promote stable and ideal breeding environments for BSF (Čičková et al., 2015; Lohri et al., 2017). Furthermore, sewage treatment can also be used as the substrate, which might guarantee the constancy of the input feed. This approach is expected to be adopted to produce prepupae biomass in large quantity and uniform quality at the commercial level (Joly and Nikiema, 2019).

Various approaches to feeding BSFL

BSFL can be reared on different feeds containing organic waste (Wang and Shelomi, 2017) and commercial feeds for the production of substantial larval and pupae biomass (Danieli et al., 2019). Processed BSFL are not required to be used in commercial feeds, as BSFL can be fed directly to birds (Logan et al., 2021). It is observed that BSFL naturally uses organic matter outside of artificial production systems.

The superlative antimicrobial peptides (AMPs) or survivability proportion of BSFL is due to the gut biome of BSFL, which is naturally prevalent in decomposing organic waste. Further research is recommended to identify the best feeding management/regime for effective production system of BSFL (Klammsteiner et al., 2021).

Feed qualities for raising BSFL

The food quality determined BSF's growth in the larval and adult stages (Nguyen et al., 2013). They are raised on a large scale organically with less cost than traditional protein sources (Meneguz et al., 2018 b). The substrate's pre-treatment activities are essential for producing BSFL. The structure of the food waste could be improved by mechanical crushing and heat treatment enabling BSF digestion (Pastor et al., 2015). The moisture content of the meal affects how well BSFL grows because saturated circumstances slow down decomposition (Cheng et al., 2017). The impact of semi-artificial lights (wavelength range between 450 and 700 nm) on BSFL growth revealed that the protein content of the larvae varied far less than the fat content (Zhang et al., 2010). Larvae also grow slowly when the food waste has a higher salt content (Cho et al., 2020). Given their ability to thrive in organic substrates with varying nutrient levels, BSFL may be able to adapt their digestive systems to satisfy their nutritional requirements.

Moreover, BSFL's mid-gut microbiota can digest a variety of substrates with variable nutrient contents, thus enhancing the ability to flourish on multiple diets (Bonelli et al., 2020). When BSFL fed on a poor diet, their digestive system's enzyme activity changed, including activity of lipase, protease and amylase. The feed conversion rate (FCR) ranges BSFL production from extremely efficient with cookie meal at 1.11 to highly wasteful with municipal organic garbage at 14.5 (Nyakeri, 2018). Dietary nutritional value has an impact on BSF growth. Animal feces and city waste showed higher FCR compared to a combination of fruit and vegetable refuse. FCR for BSFL production in the formulated diet ranges from 4.96 to 7.11 (Danieli et al., 2019)

BSF life cycle

The temperate and tropical Lepidoptera insect BSF is accustomed to mass production due to its short life cycle, big juvenile stage, high egg output, and efficiency in bioconversion. About 500–1200 eggs are laid per female (Tomberlin et al., 2002). The larvae emerge from the egg four days later and go through all six larval instars, prepupae, and pupate (Kim et al., 2010). The beige larvae have photoreceptors that enable them to escape light (Ooninx et al., 2016). Depending on the nutrition supplied and the temperature of the rearing environment, the BSF larvae take 18 to 24 days to reach the prepupal stage and weigh 300 mg (Harnden and Tomberlin, 2016; Lalander et al., 2019). A few hours later, it stops eating and starts the process of melanization, which causes the cuticle to darken before it molts to become a prepupa (Fraenkel and Bhaskaran, 1973;

Denlinger and Zdárek, 1994). The prepupa migrates to a dry location during this 7–10-day stage to transform into a pupa (Sheppard et al., 1994; Holmes et al., 2013). The pupal stage, which lasts for at least 10–30 days without movement or food, concludes with the emergence of the adult (Denlinger and Zdárek, 1994). Within its first 8 to 9 days of life, the matting of the fly starts and then lays eggs (Figure 1). The larval stages (larva and prepupa), which are the most nutrient-dense, are also the ones that BSF farmers harvest most frequently (Liu et al., 2008).

Insect processing

Harvesting is the first step in the processing of insects, which should result in a product that is secure and free from physio-chemical and microbiological properties from our point of view. Processing has a significant impact on the quality of the final product. It can improve the color and nutritional stability of the product while lowering its microbial burden. Additionally, it can alter the functional qualities of proteins and influence their nutritional quality. Processing procedures may include post-harvest processing, killing, extracting macronutrients (such as proteins, lipids, and chitin), decontaminating, drying, and grinding, depending on the insect product's ultimate use, as shown in Figure 2. Every processing step in the industry should work to improve or maintain nutritional quality while lowering the microbial load.

Harvesting

Harvesting, also known as auto-collecting, is the process of removing larvae from their eating substrate. It can be done by sieving or by utilizing their natural behaviors to allow them to gather themselves. The BSF can be harvested in two stages: larvae and prepupae. Nutritional profile depends on parameters like feeding substrate, particle size, and humidity. In the initial stage, the substrate is isolated from the larvae or prepupae using a sieve, which is rapidly used in commercial environments. Both stages can be sieved using a vibrating sieve or a rotary drum system, which allows for the collection of the substrate that could potentially serve for soil rectification (Cheng et al., 2017). To avoid blocking the filter, the substrate needs to have a minimum humidity of 50%, be well-digested, and have a particle size suitable for sieving before harvesting (Cheng et al., 2017). Auto-collection, a harvesting system that can handle high substrate humidity, only works with prepupae. This occurs because it takes the benefit of the ability to transfer to a drier area before entering the pupal stage (Sheppard et al., 1994; Diener et al., 2011 a). You can direct their population into a collection container by directing their motion down a slope caused by their effort to escape from the surface (Tomberlin et al., 2002). Post-harvest processing procedures are used in large-scale manufacturing to produce a stable insect product and to improve its quality. They might help improve the product's microbiological quality even if not all producers utilize them. They consist of a time when the feed is withheld and a washing process.

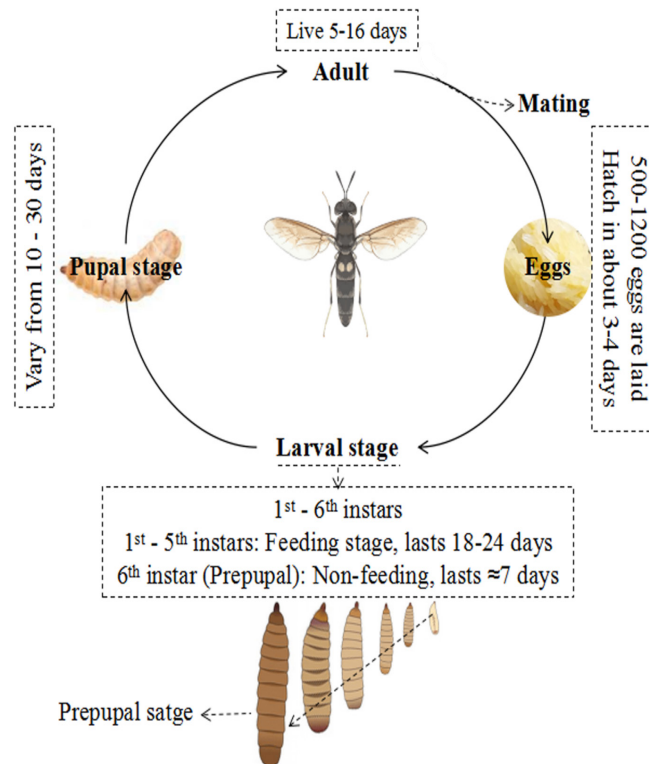


Figure 1. Life cycle of black soldier fly

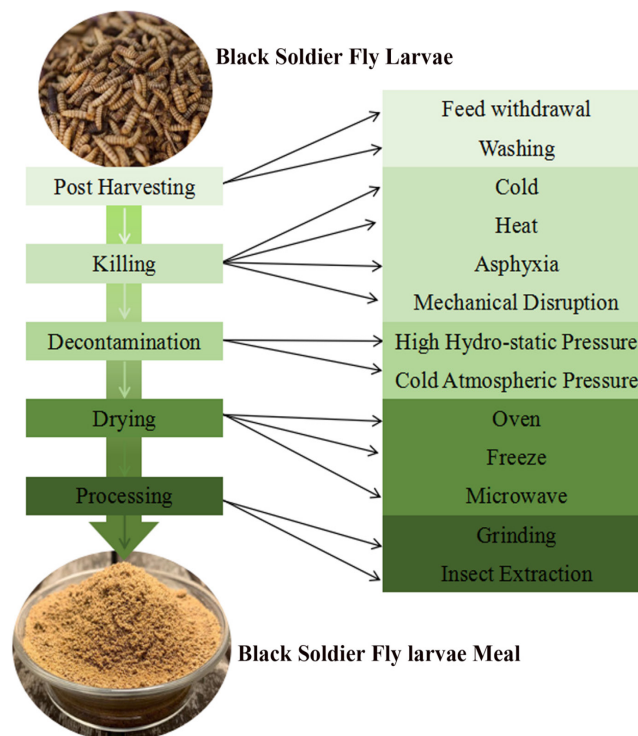


Figure 2. Processing of black soldier fly larvae for meal preparation

Feed withdrawal period

The microbial load, frass, and undigested feed they carry frequently end up in the resultant insect meal because removing the insect gastrointestinal system is challenging. Insects may be fed or degutted, which involves

exerting pressure to cause the evacuation of the contents and terminate it. To assist in decreasing the insect microbial load, the feed withdrawal period (FWP), also known as post-harvest fasting, is advised by FAO (Van Huis et al., 2013). The egestion time, defined as the amount of

time needed for a feed bolus to be expelled when feed is continually consumed, is 100 minutes and between 60 and 90 minutes (Espinoza-Fuentes and Terra, 1987; Mumcuoglu et al., 2001). When no more feed is available, the egestion time increases and is correlated with the gastrointestinal evacuation time (GET). For instance, adult *Blatella*'s GET takes 3 days, whereas egestion takes 120 minutes (Day and Powning, 1949). Insect GET is poorly understood. Thus it is widely believed that an FWP of 12 to 48 hours is sufficient to empty most of the digestive tract (Yi et al., 2013; Charlton et al., 2015). Mealworms have only been examined in case of their suitability for using an FWP to lower microbial burden (Wynants et al., 2017). The scientists concluded that mealworms' microbial load is unaffected by an FWP of up to 48 hours, with or without fecal contact (Wynants et al., 2017). Frass, however, may have persisted in the digestive tract, resulting in a continued high microbial load, as the GET of mealworms is now unclear. In addition, fasting may cause an increase in energy reserve metabolism, and assessing this metabolism is crucial for determining the ideal FWP (McCue, 2010). The efficiency of an FWP should be studied because it has the potential to lower the microbial burden at a cheap cost and because BSF larvae consume contaminated leftover organic matter.

Washing

Some insect larvae are often washed with water before being killed. Various invertebrates, such as Coleoptera (*T. molitor*) and Diptera (*M. domestica*, *Phophila casei*, and *H. illucens*), display this trait. Eliminate any feed from the cuticle before starting the operation (Stadtlander et al., 2017). Because it enables the physical removal of organic materials and any potential germs they contain cheaply and enhances self-life, washing carcasses is an efficient procedure often utilized in animal production (Dickson and Anderson, 1992). Enhancing water pressure, length, and temperature, repeatedly immersing the freight, and using sanitizers like chlorine and organic acids (acetic, propionic, and lactic acids) might enhance the washing process (Dickson and Anderson, 1992).

Killing

Killing is a crucial step in insect processing that can affect the product's nutritional value, microbiological safety, flavor, and color (Farina, 2017). Killing must be quick and efficient, which imparts to lowering the microbial burden, although retaining the nutritional value of the product and is a required step in any animal production. There are various ways to kill invertebrates, like heat, cold, mechanical disturbance, and asphyxiation. The technique needs to be modified according to the species requirement (Kouřimská and Adámková, 2016). The most prevalent techniques are freezing and blanching, although many additional techniques have the potential to be just as successful.

Killing by cold

Generally, the cold kill method performs slow killing of insects and preserves them for a longer time. Because they are poikilotherms, insects can be killed by cold, which slows down their metabolism and alleviates any possible pain or suffering. Additionally, it reduces the development of microorganisms and the action of spoilage enzymes. Killing by cold could be done by dipping it in ice water or drying it in a freezer (Fu et al., 2014). Since enzymes continue to function even after freezing, it has been observed that spoilage enzymes cause lipid breakdown and polyphenolic oxidation (Caligiani et al., 2019; Leni et al., 2019). In the BSFL, lipolysis is highly active during freezing temperature, as it enhances lipid degradation storage at -20°C (Caligiani et al., 2019). Consequently, it appears highly vital to stop such enzymes immediately to maintain insects' nutritional quality.

Killing by heat

Numerous invertebrates, including insects, lobster, and shrimp, have been killed by heat. Since blanching allows for microbial reduction while being extremely quick, it is one of the most often employed killing techniques in the production of insects (Piyasena et al., 2003). Although certain insects begin to die at 50°C, higher temperatures are typically needed to inactivate microorganisms concurrently (Armstrong et al., 2009). It can be done using moist heat, such as when steaming and blanching food, or dry heat, like in an oven. Heat treatment can stimulate the denaturing of endogenous enzymes that cause spoilage of nutrients during storage and lower the insects' microbial burden (Manheem et al., 2012). Blanching for BSFL is lower due to the higher water content (Ames, 1990). Shrimp's protein and water content have reportedly been reduced by immersion techniques, including blanching, which could speed up drying time (Niamnuy et al., 2008).

Asphyxia

One of the most widely used techniques for anesthetizing invertebrates is carbon dioxide (CO₂), which needs to be exposed for anywhere between 3–60 minutes, depending on the type of insect (Nicolas and Sillans, 1989). Insects become immobile when CO₂ concentrations rise above 40% without affecting their permeability (Tonnejck-Srpová et al., 2019). However, it can potentially be fatal when used for an extended time since it lowers the hem lymph's pH and causes dehydration by encouraging spiracle opening (Woodring et al., 1978; Wong-Corral et al., 2013). Additionally, oxygen is released due to lowering its metabolic activity, which extends their duration under anoxic conditions (Cheng et al., 2013). However, the metabolism activity is sustained in the presence of CO₂, causing oxygen depletion and lowering their chance of survival (Cheng et al., 2013). The time needed to kill insects with CO₂ varies greatly depending on different parameters like humidity, oxygen and carbon dioxide concentration, temperature, insect species, and its stage

(Husain et al., 2017). For *Drosophila melanogaster* (Diptera) larvae, pure CO₂ is fatal in only 30 minutes (Badre et al., 2005), whereas it takes 72 hours to kill larvae of *Cadra cautella* (Lepidoptera) and *Tribolium castaneum* (Coleoptera) (Badre et al., 2005). Even though CO₂ asphyxiation takes a while, it can anaesthetize the insect before killing it and speed up drying by starting the dehydration process resulting from spiracle opening.

Asphyxiation could be done with saturated N₂, but oxygen content must be below 3% (Hashem et al., 2014). Species and developmental stages of insects differ in their ability to survive hypoxia. In addition to being highly varied, the death of larval stages of the lepidopteran *Ephesia castella* to 98% N₂ needs between 96 and 144 h, or 1 to 2 days longer than an aerobic environment with 60% carbon dioxide (Hashem et al., 2014). Research on the anoxic tolerance of BSF larvae is scarce, although they may have a similar risk to 100% CO₂ as *D. melanogaster*. Prolonged anaerobic conditions might promote the growth of anaerobic bacteria, demanding a thorough disinfection.

Mechanical disruption

The quickness and low cost of mechanical disruption of the neural system seem like a potential killing technique. According to Protix (a multinational manufacturer and supplier of insect ingredients for animal feed and human consumption, Netherlands), it can be administered through centrifugation or grinding. Mechanical killing seems to be used in industry, as it has not been studied and examined how it affects the final product's quality (Ouellet, 2017). According to reports, grinding causes browning in a variety of insect species. By exposing the components of insects to oxygen, it might also enable lipid oxidation (Barden and Decker, 2016).

Additionally, grinding could increase surface evaporation, increasing drying capacity (Barden and Decker, 2016). A wide range of techniques are needed for insect-killing, but only freezing and blanching must be considered. As a result, it is essential to refine how BSFL are killed to increase its effectiveness while lowering potential suffering.

Decontamination

The finished product is a complete wet larva that can be killed. Depending on the method used to kill it, its microbial load may have been lowered, and its pH is nearly neutral (Wynants et al., 2017), indicating that it is a perishable product that should be treated as a low-acid food (Larouche et al., 2019). The larvae must consequently be processed to increase their shelf life. Many techniques have been tested for lowering the microbial load of insects (Sun et al., 2017). High hydrostatic pressure, microwaves, blacking, dehydration, and direct and indirect cold plasma are utilized (Kashiri et al., 2018).

High hydrostatic pressure

A non-heating decontamination method known as high hydrostatic pressure (HHP) technology can retain

most of the product's value identified while lowering its microbial burden. In this process, the product enters a room filled with a compressible fluid in a batch system, and then the air is removed. The product then experiences an instantaneous, uniform pressure due to the fluid being compressed, either directly or indirectly, allowing the product to keep its structure (Muntean et al., 2016). During pressurization, a high temperature may be provided to boost effectiveness (Holmes et al., 2013). The applied pressure (MPa), pressure holding time, and temperature could be tuned to improve therapy effectiveness (Holmes et al., 2013).

High hydrostatic pressure is widely used in food processing, although it is still quite expensive. Depending on the conditions, this technology has some advantages over conventional techniques at low temperatures, including the ability to pressurize foods, a reduction in load of some bacteria, yeast, and molds, inhibition of enzyme activity, and the ability to develop new functional foods (Gao and Jiang, 2005; Keenan et al., 2012; Muntean et al., 2016). However, high pressure could significantly reduce gram-negative bacteria, yeast, and mold, although spore forming bacteria and gram-positive bacteria are less significantly reduced. According to studies, the existence of spore-forming bacteria remains unaffected by the treatment, causing HHP's effects on total viable counts (from 1.0 to 8.0 log reduction) when administered to insects (Salvatierra Contreras, 2017). Hence, HHP needs to be adjusted according to the microbiome of the product, which differs based on insect species, life cycle, and processing circumstances. Hydrostatic pressure has been shown to reduce the water retention ability of mealworms, with a more significant impact observed at higher pressures (Tonnejck-Srpová et al., 2019). Reduced drying time could potentially be a benefit for the industry due to lower water-holding capacity. HHP can, however, also have detrimental effects on product quality since it can encourage lipid peroxidation (Medina-Meza et al., 2014), as was noted that lipid oxidation levels rose from 300 MPa for gigantic tiger shrimp (Kaur et al., 2016). It is necessary to assess the potential efficacy of that technique for insects, particularly BSF larvae, considering the diverse impacts of HHP.

Cold atmospheric pressure plasma

A non-thermal method called cold atmospheric pressure plasma allows for the reduction of various microorganisms, including viruses and bacteria, that can generate spores. Although interest in it is growing, it is pricey (Bußler et al., 2016). The assault of cold plasma's neutral and sparsely charged particles renders bacteria inert by dissolving their cell membranes (Hertwig et al., 2018). As a result, this therapy is less effective against gram-positive bacteria and spores (Hertwig et al., 2018). Cold plasma administered directly or indirectly to fresh mealworms has a minimal impact (Rumpold et al., 2014), but when applied semi-directly to powder, it may lower total viable numbers by 3 logs (Bußler et al., 2016).

Other decontamination techniques, like heat treatment, microwave, and pulse electric field, can be utilized to lessen the microbial burden on insects (Hertwig et al., 2018). Heat and microwave treatment can be applied to insects to render germs inactive. The microbial burden could be significantly reduced by microwaving. There are two types of heat treatment: wet heat and dry heat (desiccation heat). Most microorganisms can be significantly reduced by blanching for only 40 seconds in water. However, spore-forming bacteria are unaffected (Vandeweyer et al., 2017). As aerobic endospores blanching, it can be considered an efficient method that works as an acidic solution (Klunder et al., 2012).

Drying

Drying is a technique used for the preservation by lowering microbial growth and spoilage-enzyme activity with several other benefits (Huang et al., 2019). Drying insects is possible using various techniques, each of which will change how the dried result turns out. The technique can impact the color, water activity, and protein solubility (Janssen et al., 2019). The most popular techniques for processing insects include sun, oven, freeze, and microwave drying.

Oven drying

It has been demonstrated that oven drying holds much promise as an insect drying technique (Fombong et al., 2017; Huang et al., 2019). Vacuum and rotating ovens are just two examples of several systems that can be employed, and each had a different impact on the final product (Kröncke et al., 2018). A lower temperature is preferable to retain protein solubility and to minimize the Maillard reaction, shrinkage, and tissue collapse (Kröncke et al., 2018; Melis et al., 2018; Purschke et al., 2018). Temperatures between 50 and 120°C are often administered for an hour to a few days. The most effective drying temperature to avoid these effects and shorten drying times is 60°C (Azzollini et al., 2016).

Freeze-drying

Mealworms must be dried using freeze-drying, a non-thermal procedure that is often used in insect processing, but it is relatively expensive and takes at least 24–53 hours (Kröncke et al., 2018; Lenaerts et al., 2018). Since it does not cause the Maillard reaction, which produces the whitest powder, it is one of the finest drying techniques for maintaining insect color (Lenaerts et al., 2018). However, it also causes a modest 10% drop in protein solubility (Kröncke et al., 2018). Furthermore, freeze-drying results in the most extensive oxidation of lipids, which significantly reduces quality, while blanching before freeze-drying decreased oxidation by half (Lenaerts et al., 2018). Additionally, because all freeze-dried larvae appear expanded rather than shrunk, it can increase consumer acceptance of BSF larvae.

Microwave drying

Microwave drying takes only 10 to 15 minutes, but is expensive (Lenaerts et al., 2018). It generates inflated, entire dried larvae, but permits browning (Huang et al., 2019). Mealworm protein solubility was 40% lower after microwave drying (Kröncke et al., 2018). Compared to oven drying at 60°C, microwave drying on BSF larvae stimulates protein polymerization, resulting in a powder with larger particle sizes (Huang et al., 2019).

Insects may also be dried using other techniques, such as fluidized beds and the sun. Sun drying is a time-honored, traditional, low-cost technique used to treat various insect species, including grasshoppers, caterpillars, termites, and maggots (Melis et al., 2018). Since fluid bed drying only slightly increases lipid oxidation when applied at 130°C for 110 min, it can also be employed as a quick, high-temperature, short-time method (Kröncke et al., 2018). Most investigations on edible dried insects centered on *T. molitor*, and few others have investigated how drying affects the quality of BSF larvae or prepupae (Kröncke et al., 2018). Therefore, a deeper comprehension of how drying affects the BSF may help enhance the finished product's quality.

Processing

The product becomes stable after being decontaminated and dried and should be stored for at least a few months without significantly losing its nutritious quality (Sprangers et al., 2017 b). Since the product is currently stable enough to ship and store, additional processing stages are unnecessary, although they could add value or improve the product's quality (Mutungi et al., 2019).

Grinding

Generally, edible insects are used in a diet as powder. Grinding can seem incredibly appetizing. If insects are used in food but cannot be seen, they are more likely to be accepted by Western consumers (Tan et al., 2015) if used as a powder in a dish. Nevertheless, it has been noted that grinding fresh insects can cause toasting in many insects due to enzymatic and non-enzymatic reactions involving polyphenols, which may lower product quality (Yi et al., 2013; Janssen et al., 2017). Therefore, grinding insects must be done only when the product has reached a stable state.

Extraction of insect components

The product's value may be increased by the extraction of lipids, proteins, and chitin by tighter control over the percentage of their presence in diet composition. Conversely, to enhance extraction outcome with purity. The extract can change the flavor, the color, and the functioning of the protein, cause protein denaturation, and lower the yield of protein extraction from the remaining defatted meal (Swamylingappa and Srinivas, 1994; Tzompa-Sosa et al., 2014). Mechanical pressing, aqueous or solvent extraction, and differential density are the methods for removing lipids from a sample. Alkali or

sonication are two standard methods for protein extraction from defatted insects (Caligiani et al., 2018; Mishyna et al., 2019). Finally, chitin extraction involves demineralizing the product and then deproteinizing it. This can be accomplished with chemicals or lactic acid bacteria.

Additionally, new extraction technologies such as supercritical CO₂, microwaves, and ultrasound should be further studied (Chemat and Khan, 2011; Purschke et al., 2018). HHP and blanching are two processing methods that can improve component extractability (Barba et al., 2015). Therefore, processing techniques should be considered whole, beginning with harvest and ending with the desired final product. In fact, the various processing techniques mentioned above can influence the final product's features in a variety of ways, enabling the reduction of the required processing steps or an improvement in the product's quality.

Nutritional value of BSFL

Regular nutrition facts

Currently, two types of BSFL are used in animal feed: defatted and full-fat fat. The main distinction between the two is the amount of fat and saturated fatty acids in them (Table 1). Crude protein contents of BSFL meal (216 to 655 g/kg) are comparable to those of fish meal (675.3 g/kg) and soybean meal (SBM) (494.4 g/kg) with an average value of 414.7 g/kg. A full-fat BSFL meal has roughly similar protein content. However, the protein content after defatting differs significantly (216 to 655 g/kg), which could be because of the defatting process, which may be linked to the defatting process like a permanent disintegration of protein brought on by high temperatures (Queiroz et al., 2023). The fat content of full-fat BSFL meal ranged from 294 g/kg (Onsongo et al., 2018) to 515.3 g/kg (Tyshko et al., 2021), with an average of 353.2 g/kg. These values are greater than those of SBM (14 g/kg) and fish meal (103.6 g/kg).

The average fat content (69.2 g/kg) is higher than the defatted SBM. BSFL contains about 82.4 g/kg of ash on average, which is higher than SBM (71.9 g/kg) but lower than fish meal (171.5 g/kg) (Council, 2012), ranging from 27 g/kg to 132 g/kg (Onsongo et al., 2018). The crude fiber content of BSFL ranges from 41 g/kg to 213 g/kg, with 95.4 g/kg on average (Onsongo et al., 2018). The BSFL contains about 38.7 g/kg to 72.1 g/kg of chitin, averaging 61.7 g/kg (Lu et al., 2022). In addition to cellulose, chitosan is another significant polysaccharide and the active component of chitin. Similar molecular configurations can be found in chitin, cellulose, a linear polymer comprising (1–4) D-glucopyranose units and (1–4) N-acetyl-D-glucosamine units (Finke, 2007). Although chitin is regarded as an indigestible fiber, animals' immune systems can benefit from it (Li et al., 2013; Swiatkiewicz et al., 2015).

Amino acid profile

Both defatted or full-fat BSFL contain an enormous amount of amino acids compared to the fish or soybean meal (Crosbie et al., 2020). Table 2 displays the BSFL amino acid profile. Leucine was the most prevalent amino acid (on average). Lysine, valine, and leucine are three essential amino acids with higher concentrations in BSFL meal than in soymeal meal. Moreover, valine concentration is higher in BSFL than in fish meal (Council, 2012). The least essential amino acids like tryptophan and methionine in BSFL are comparable to soybean meal, but significantly lower than fish meal (Li et al., 2021). Histidine and isoleucine contents were slightly more significant than those of soybean meal and fish meal (Eugenio, 2022). Phenylalanine levels varied from 16.4 to 77.6 g/kg, and threonine content varied from 16.2 to 45 g/kg, identical to fish meal and soybean meal. Meanwhile, histidine and arginine values are lower in fish meal and soybean meal, respectively (Yaghoubi et al., 2016).

Table 1. Comparative nutrition facts of BSFL with SBM and FM (g/kg dry matter basis)

Type	BSFL									SBM	FM
	FF	DF	DF	FF	FF	FF	FF	FF	DF		
Chitin	67	69	N/A	N/A	N/A	N/A	N/A	38.7	72.1	N/A	N/A
Ash	27	93	93	93	132	53	104	65.9	81	71.9	171.5
Crude fiber	41	N/A	70	N/A	213	79	N/A	N/A	74	74.3	2.6
Crude fat	386	46	63	301	294	298	325	515.3	98.5	14	103.6
Crude protein	431	655	216	411	439	350	401	275.4	554.2	494.4	675.3
References	(Sprangers et al., 2017 b)	(Schiavone et al., 2017 a)	(Yildirim-Aksoy et al., 2020)	(Shumo et al., 2019 c)	(Onsongo et al., 2018)	(Rawski et al., 2020)	(de Souza Vilela et al., 2021)	(Tyshko et al., 2021)	(Tyshko et al., 2021)	(Council, 2012)	(Council, 2012)

SBM = soybean meal, FM = fish meal, FF = full-fat, DF = defatted.

Table 2. Comparative amino acid profile of BSFL meal with SBM and FM (g/kg dry matter basis)

	BSFL meal							SBM	FM	
	Type	FF	DF	FF	FF	FF	FF			
References	Indispensable amino acids									
	Arginine	19.9	20.7	21.1	54.7	62.0	21.9	18.7	35.7	41.0
	Histidine	13.8	16.3	13.5	32.5	48.0	9.8	13.7	14.2	15.4
	Isoleucine	19.1	24.0	17.7	47.3	48.0	19.1	20.6	22.1	27.3
	Leucine	30.6	36.7	27.8	78.3	77.0	32.1	29.4	38.6	47.7
	Lysine	23.0	25.2	28.1	68.2	74.0	27.2	25.9	31.1	48.7
	Methionine	7.1	8.56	8.0	21.2	6.0	6.0	7.1	6.8	18.5
	Phenylalanine	16.4	21.8	16.4	77.6	62.0	18.3	18.7	25.5	26.4
	Threonine	16.2	21.8	16.3	44.3	45.0	26.5	16.7	19.8	27.5
	Tryptophan	5.4					5.6	6.3	6.6	6.7
	Valine	28.2	34.5	25.0	67.9	67.0	28.7	28.8	21.7	32.7
	Dispensable amino acids									
	Alanine	27.8	43.7	25.6	82.1	62.0		26.6	21.6	41.9
	Aspartic acid	36.9	48.8	38.7	73.0	103.0		35.6	55.0	57.7
	Cysteine	2.2	0.2	3.5	7.6	5.0	4.2	3.2	7.7	6.5
	Glycine	25.2	30.3	24.6	61.5	54.0	26.8	24.8	21.3	50.3
	Glutamic acid	45.8	63.7	46.1	131.0	102.0		38.4	88.6	84.1
	Proline	25.1	32.7	23.6	66.8	62.0		23.1	27.4	30.8
	Serine	15.9	26.8	17.6	48.8	41.0	19.2	15.2	24.1	25.9
Tyrosine		34.1		67.1	60.0	26.5	26.9	15.5	20.1	
	(Sprangers et al., 2017 a)	(Schiavone et al., 2017 a)	(Onsongo et al., 2018)	(Rawski et al., 2020)	(Müller et al., 2017)	(Shah et al., 2022)	(Romano et al., 2022)	(Council, 2012)	(Council, 2012)	

SBM = soybean meal, DF = defatted, FM = fish meal, FF = full fat.

Fatty acid composition

Lauric acid (C12:0) is the most abundant saturated fatty acid (SFA), with a concentration ranging from 75 to 575. The concentration of myristic acid (C14:0) ranges from 23 g/kg to 98.7 g/kg, palmitic acid (C16:0) ranges from 10.3 g/kg to 192 g/kg, and stearic acid (C18:0) ranges from 9.8 g/kg to 69 g/kg. Oleic acid (c9C18:1) is the most abundant monounsaturated fatty acid in amounts ranging from 79.7 to 266 g/kg. Concentrations of palmitoleic acid (C16:1), linoleic acid (C18:2n-6), and linolenic acid (C18:3n-3) range from 8 g/kg to 33.4 g/kg, 38 g/kg to 314 g/kg, and 9.8 g/kg to 36 g/kg, respectively. MUFA content ranges from 85.5 g/kg to 287 g/kg, while SFA concentration varies from 362 g/kg to 782.9 g/kg. n-3 PUFAs range from 9.8 g/kg to 36 g/kg, and -6 PUFAs range from 80 g/kg to 314 g/kg. The range for n-3 PUFA to n-6 PUFA is 8.5% to 17.9%, which is more variable compared to the range of 15.3% to 79.3% for MUFA to SFA (Lu et al., 2022), as presented in Table 3.

Composition of minerals

Calcium is the most prevalent mineral in BSFL meal and is present in high concentrations ranging from 1.2 to 35.7 g/kg. The range of copper and iron is between 0.1 and 15.0 g/kg and between 0.1 and 191 g/kg, respectively. However, magnesium content ranges from 1 to 3.5 g/kg, and manganese content ranges from 0.2 to 166 g/kg. The phosphorus is 1.0 to 10.3 g/kg, the potassium phosphorus range is 1.0 to 10.3 g/kg, and the potassium range is 1.7 to 15.4 g/kg. The range of sodium is 0.7 to 15.6 g/kg, while the zinc range is 0.7 to 103 g/kg. Some perilous and hazardous substances like barium, mercury, and molybdenum are also present in BSFL (Table 4). Moreover, these toxic minerals could also be involved in BSFL metabolism (Bulak et al., 2020) and could become a critical challenge for the safety of feed and food production (Petlum et al., 2019).

Table 3. Fatty acid composition of full-fat BSFL meal

Fatty acids	Concentration (g/kg dry matter)					
C10:0	20.3		8.6	14.3		8.6
C12:0	575.6	75.0	459.7	526	468.6	407.9
C14:0	71.4	23.0	87	85.4	98.7	65.6
C15:0			1.5		143.8	1.3
C16:0	10.3	192.0	122.1	109	143.8	162.7
C18:0	9.8	69.0	25.3	15.3	17.9	14.3
SFA	782.9	362.0	707.2	750.0	742.4	664.2
C16:1	33.4	8.0	19.1	19.8	27.8	23.6
c9C18:1	79.7	266.0	112.4	61.6	77.3	182.4
c11C18:1	1.2			2.4		
MUFA	119.9	287.0	134.1	85.5	115.8	218.8
C18:2n-6	78.3	314.0	38.0	116.0	127.7	100.7
n-6 PUFA	80.0	314.0	142.2	119.0	106.0	100.9
C18:3n-3	11.0	36.0	16.5	10.1	9.8	16.0
C18:4n-3	0.5					
C20:5n-3	2.3					0.2
C22:6n-3	0.1					
n-3 PUFA	14.3	36.0	16.5	10.1	9.8	16.2
MUFA/SFA, %	15.3	79.3	18.9	11.4	15.6	32.9
n-3 PUFA/ n-6 PUFA, %	17.9	11.5	11.6	8.5	9.0	16.1
References	(Spranghers et al., 2017 b)	(Ewald et al., 2020)	(Daszkiewicz et al., 2022)	(Rawski et al., 2020)	(Nayohan et al., 2022)	(Rabani et al., 2019)

MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids.

Factors affecting the nutritional composition of BSFL meal

The mineral and nutrient content of BSFL meal varies between studies, and the factors mentioned in Figure 3 could be the reason.

1. BSFL may go through distinct growth stages. The crude fat content of larvae increased significantly during the developing stage between days 4 and 14, reaching a maximum of 28.4%. Meanwhile, crude protein continued to decline simultaneously; however, the crude fat content quickly decreased to 24.2% as pupa grew. Adult BSFL has the utmost protein (57.6%) and fat (21.6%) content (Liu et al., 2017).
2. The fat and ash content obtained from vegetable waste, poultry feed, and kitchen waste changes significantly concerning the nutritional composition consumed by BSFL (Tschirner and Simon, 2015). Additionally, BSFL on cow dung increases in size at a considerably slower rate than on poultry feed (Diener et al., 2009; Myers et al., 2014).
3. The technique of processing might be relevant. The pH, ash, lipid content, and oxidative capability could be

- impacted by various killing techniques (including high hydrostatic pressure, grinding, drying, freezing, and asphyxiation) (Larouche et al., 2019; Purba et al., 2021). The nutritional value of BSFL meal is also impacted by the temperature and storage technique (Saucier et al., 2022). The nutritional composition of various extraction processes varies as well. For instance, alkali extraction produces the best separation of protein (Caligiani et al., 2018). The BSFL processing at 25°C could provide up to 7 months of shelf life (Kamau et al., 2018).
4. It depends on several variables: temperature, humidity, sunshine, moisture content, pH, etc. The incubation, development, and lifetime of BSFL will be impacted by humidity and temperature (Tomberlin et al., 2009; Chia et al., 2018). The best rearing conditions for BSFL are temperatures of 26–40°C and relative humidity levels of 40–70% (Sheppard et al., 2002; Holmes et al., 2012). The nutritional makeup of BSFL is similarly influenced by sunlight, with BSF flourishing best in the 450–700 nm wavelength range (Zhang et al., 2010). The water content of the feed matrix between 60 and

80% exhibited the higher survivability and growth rate of BSFL (Banks et al., 2014). SBF growth is more favorable in alkaline than in acidic environments, and a desirable pH range is between 6 and 9 (Ma et al., 2018; Meneguz et al., 2018 a).

BSFL as animal feed

Sustainable recycling technology transforms organic waste into larvae or prepupae utilizing BSFL (Diener et al., 2011 a). The nutritional profile of BSFL utterly depends on the age and substrate that makes them an ideal nutrient source for animal feed because of more significant protein and fat content (Kim et al., 2010; Spranghers et al., 2017 b). BSFL can be fed to animals either live or processed. In the standard typical feed composition, BSF larvae or pupae-derived protein exhibited equivalent performance as soybean or meat meal (Čičková et al., 2015). Among the ten essential amino acids that animals must consume in their diets are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Halver, 1957, 1960; Shumo, 2019 a). The amino acid content of proteins affects the quality of animal feed (Müller et al., 2017) – the amino acid content of BSFL changes according to the substrate. BSFL raised on kitchen waste increased the total amino acid content (Shumo, 2019 a). From BSFL meal, methionine is an amino acid that did not satisfy FAO recom-

mendations (2013), while the rest are marginally above the permissible level. The amino acids in BSFL meal are comparatively more essential than other conventional protein sources used for animal feed (Table 5). Chiefly, the BSFL meal had a higher amino acid concentration than soybean meal and soy concentrate. Additionally, as a protein ingredient, it compares favorably to fish meal and soy protein.

Economic analysis showed two main financial advantages of the BSF technology: the comparatively low cost of treating organic waste and the income from the sale of high-value protein derived from larvae (Diener et al., 2009; Spranghers et al., 2017 b). The industry constantly seeks alternative protein sources to supplement or replace traditional feeds due to the rising need for animal feed. BSFL meal hence has a large market. In response, insects have gained interest as a feed source, with BSFL being one of the most favorable insect species for artificial raising, farming, and the manufacturing of industrial feed (Sripontan et al., 2017). The global feed production requirement was more than 1 billion tons in 2017, according to the International Feed Industry Federation, and BSFL meal has a potential market value of USD 400 billion (Joly and Nikiema, 2019). Therefore, BSF production's economic viability is proposed to have significant worldwide market potential by the rise in pricing for traditional animal feed (Lalander et al., 2019).

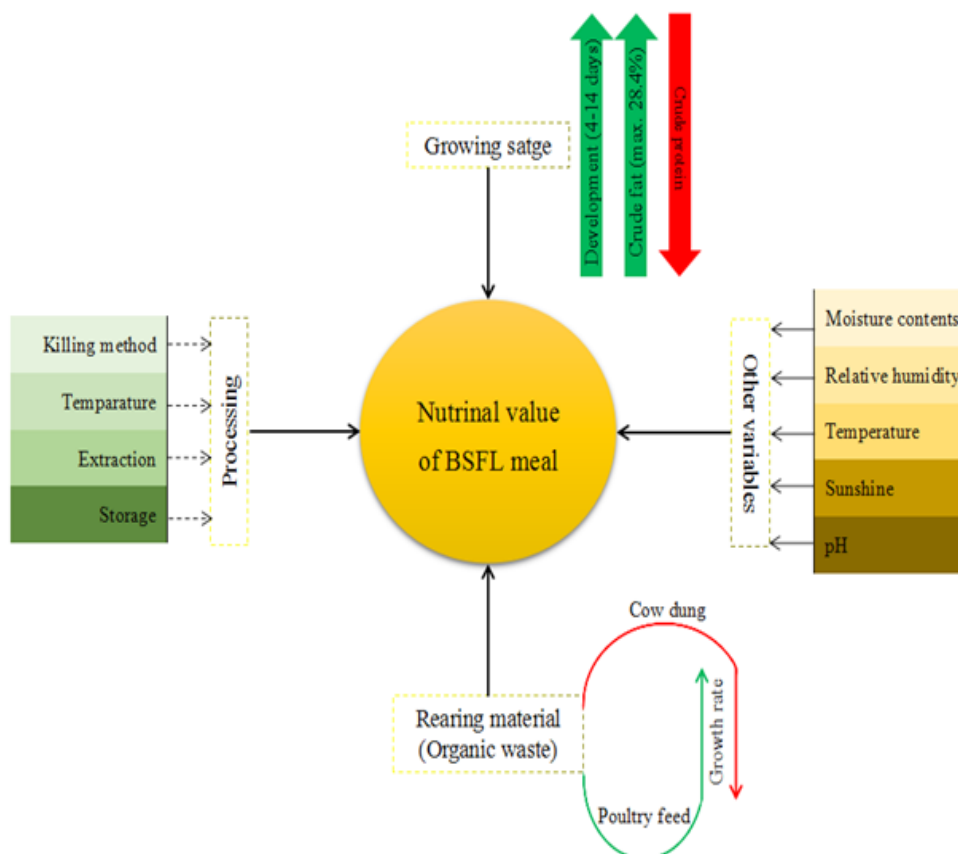


Figure 3. Factors affecting the nutritional value of BSFL meal

Table 4. Mineral compositions of BSFL meal (g/kg dry matter basis)

Minerals	FF	DF	FF	FF	FF
Calcium	1.2	13.0	1.9.0	34.6	35.7
Copper	0.1	15.0	0.6	10.7	0.7
Iron	0.1	125.0	2.1	191.0	14.0
Magnesium	2.1	3.0	1.0	3.5	3.4
Manganese	0.2	45.0	0.3	166.0	33.5
Phosphorus	4.1	8.0	1.0	10.3	7.0
Potassium	6.0	11.0	1.7	15.4	9.2
Sodium	0.7	5.0	3.3	1.7	15.6
Zinc	0.7	90.0	0.9	103.0	9.0
References	Sprangers et al., 2017 b	Yildirim-Aksoy et al., 2020	Shumo et al., 2019 c	Shah et al., 2022	Smets et al., 2020

DF = defatted, FF = full fat.

Table 5. Comparison of essential amino acid content (mg/g) of BSFL meal with other protein sources and FAO recommendation (2013)

Amino acid	Soybean meal	Whey protein	Soy concentrate	Fish meal	BSFL meal	FAO (2013)
Arginine	26	21	46	63	62	–
Histidine	10	22	16	20	48	16
Isoleucine	17	58	29	37	48	30
Leucine	27	102	49	65	77	61
Lysine	22	96	39	69	74	48
Methionine	5	19	8	26	6	23
Phenylalanine	18	33	33	33	62	41
Threonine	15	72	25	39	45	25
Tryptophan	5	21	8	9	–	6.6
Valine	17	58	31	45	67	40
Reference	DiGiacomo, 2019		Müller et al., 2017		Wang, 2021	

Effect of feeding BSFL meal in poultry

Growth performance

BSFL meal was a promising protein source for poultry and exhibited the exemplary results when the inclusion level was 10%; however, 100% BSFL meal resulted in adverse effects on growth performance and elevated abdominal fat deposition. Studies conducted by Maurer et al. (2016) and Murawska et al. (2021) observed weak growth performance when 100% soybean meal (SBM) was replaced with full-fat BSFL meal. 100% full-fat BSFL meal as a replacement for SBM reduced the body weight (BW) and the average daily gain (ADG) (Maurer et al., 2016; Murawska et al., 2021). However, Berger et al. (2021) observed decreased average daily feed intake, muscle weight, carcass weight, and feed conversion ratio and increased abdominal fat deposition with the increasing inclusion of BSFL. Numerous studies suggest that 10% BSFL meal is the optimum level and has the potential to enhance growth performance. 10% BSFL meal in broiler chicken's diet significantly improved the BW, ADG, ADFI, and FCR (Dabbou et al., 2018; Onsongo et al., 2018). Moreover, Kawasaki et al. (2019) and Schiavone et al. (2018) observed a non-significant effect of a 10% BSFL meal diet on BW, ADFI, ADG, eggshell

weight, liver weight, carcass weight, breast, heart, thigh, spleen, and abdominal fat of broiler chicken and laying hen. However, inconsistent studies showed that BSFL meal supplementation in laying hens' diets had no impact on their FCR, DFI, FCR, LW, breast, or abdominal fat (Onsongo et al., 2018; Gariglio et al., 2019; Bellezza Oddon et al., 2021; Murawska et al., 2021). These studies suggest that 10% BSFL meal inclusion effectively boosts the broiler chicken's growth performance; however, its impact on laying hen and complete SBM replacement needs further investigation.

Biochemical and immunological performance

The effects of BSFL meal on different blood biochemical and immunological parameters of poultry are described in Table 7. BSFL meal at 5 to 15% in poultry diet improved the glutathione peroxidase (GPx) levels and overall antioxidant status (TAS); however, it did not affect the cholesterol, creatinine, heterophil to lymphocyte ratio, urea acid, leukocyte, and aspartate aminotransferase (AST) (Dabbou et al., 2018). Similar findings stated that 3 to 9% BSFL meal added to chickens' diet had no impact on their erythrocyte, heterophil, lymphocyte, basophil, AST, alanine aminotransferase (ALT) or γ -glutamyl transferase (GGT)

levels (Gariglio et al., 2019). Moreover, BSFL meals possess antioxidant properties (Sypniewski et al., 2020). 9% BSFL meal in the diet decreased the plasma malondialdehyde (MDA) and nitrotyrosine levels compared to the control; however, cholesterol concentration decreased from 30.23% to 23.86%. In addition, 100% BSFL in broiler's diet and 5% in hen's diet exhibited no negative impact on biochemical and immunological health (Schiavone et al., 2017 b; Loponte et al., 2017; Bellezza Oddon et al., 2021); however, Marono et al. (2017) reported a slight variation. A contrary study shows that 100% of the replacement of BSFL meals with soybean meals raised the triglyceride, globulin, and cholesterol levels without affecting immunological markers (Yu et al., 2023). BSFL meal in duck diet did not vary the TAS, GPx, MG, AST, and ALT levels; however, MDA fell linearly as BSFL intake increased (Siddiqui et al., 2024). Furthermore, BSFL meals in chicken diets increase the circulating immunoglobulins in the globulin-to-albumin ratio, thus enhancing disease resistance and immune function without adverse effects (Gariglio et al., 2021). These findings suggest that 3–15% BSFL meal in poultry diets enhances their antioxidant status and potentially boosts immunity without compromising overall health. In order to understand the impact of BSFL meals on the immunological and biochemical responses of poultry species, more research is required to determine the optimal levels of BSFL as a feed supplement for poultry and how it can positively influence their health and well-being.

Meat quality and nutritional content

The impact of the BSFL meal on the nutritional content and quality of the chicken meat is demonstrated in Table 8. When BSFL meal was substituted for soybean meal, there were no significant differences in meat moisture, protein, lipid, ash, cholesterol, thiobarbituric acid reactive substances, pH, and meat color (L^* , a^* , b^* value). Including a 10% BSFL meal in the diet showed a steady increase in the FA/PUFA ratio, decreased yellowness and muscle hydration, and increased redness and muscle protein content (Cullere et al., 2019). However, BSFL meal increased FA content from 33.8% to 36.7% (Moula et al., 2018). Numerous investigations observed a significant increase in muscle pH and yellowness with increasing levels of BSFL meal inclusion, >75% and 50% replacement of SBM, respectively; however, no influence on the sensory characteristics like tenderness or aroma was observed (Murawska et al., 2021).

A study by Schiavone et al. (2019) showed that 10% BSFL meal increased a^* and the protein content of muscle, and 15% BSFL meal lowered b^* and moisture of muscle; however, the FA/PUFA ratio increased with the increasing inclusion level of BSFL meal (Schiavone et al., 2019). The increase in meat redness could be caused by an accumulation of pigments from the insect meal; however, meat yellowness could be a result of the high concentration of corn gluten in the diet. Moreover, the fatty acid (FA) profile of BSFL meal directly influences the FA composition

of poultry meat (Altmann et al., 2018). Diets containing 10 to 15% BSFL increased aspartic acid, glutamic acid, alanine, serine, tyrosine, and threonine levels in muscle tissue, which indicates that poultry can effectively utilize and retain amino acids derived from BSFL feed (Cullere et al., 2018). In another research, BSFL meal did not influence 24 h pH or thigh and chest muscle colors; however, increasing the inclusion level of BSFL meal significantly increased the lauric acid (C12:0), myristic acid (C14:0), and linolenic acid (C18:3 n-3) levels (Gariglio et al., 2021). These studies suggest that BSFL in chicken's diet has the potential to enhance overall meat quality by improving meat color, FA profile, and essential amino acid content. Including meat rich in essential nutrients in our diet can play a significant role in maintaining good health. It provides the body with vital vitamins and minerals necessary for optimal functioning of human systems. Although BSFL meal has shown potential as a sustainable protein source for poultry, more research is required to determine the optimal level of BSFL inclusion and its long-term impact on meat quality. Additionally, further investigation is necessary to understand the potential health benefits of consuming meat from animals fed with BSFL.

Other beneficial uses

BSFL as a source of biodiesel

BSFL prepupae has a high content, providing a significant portion of the nutrients and serving as a desirable biodiesel production feedstock. In an experiment, fat is extracted from larvae by altering petroleum ether through the esterification of free fatty acids with an acid catalyst (to reduce the acidity of crude fat) and esterification with an alkaline catalyst. The BSFL fat can be used to make biodiesel after extraction. Compared to current biodiesel sources, BSFL is more efficient at using the land because it requires less space, takes less time to produce, and offers superior fertility than palm oil or sugar cane (Li, 2011). The biodiesel quality depends on the lipid quality of BSFL (Giannetto, 2020). Compared to the European Biodiesel Standard (EN14214), the fat-based biodiesel produced by BSFL has an advantage because its fuel quality meets the standards. The oil obtained from BSFL was less suited than locust or cricket oils for skincare products due to the high level of lauric acid, which is more than 60% of the BSFL fatty acid profile (Verheyen, 2018). Enhancing the marketability of these insect derived oils (BSFL, locust and cricket) may require evacuation of odor, color, phospholipid, and free fatty acid content. Prior research indicates that BSFL is good-quality biodiesel (Zheng et al., 2012). Despite a modest increase in acid value from EN14214, most properties fall within the range. Although BSFL meals have high lipid content and are not commonly used in food production in many Asian nations, they can be used as a substitute for sugar cane and corn in biodiesel production (Kim et al., 2021). Literature advocates that BSFL is a promising alternative for biodiesel production due to its high lipid content, short life cycle and efficient land use; however, some properties need to be optimized to enhance its marketing.

Table 6. Comparative effects of replacing conventional protein sources with BSFL meal on different performance parameters in poultry

References	Breed	Age (Days)	Type	BSFL meal, %	Comparative effect on different parameters as compared to control group							
					DFI	ADG	LW	FW	FCR	Abdominal fat %	Breast weight	Mortality (%)
Onsongo et al., 2018	Cobb 500	1-79	FF	5-15	-	-	-	+	-	-	-	-
Murawska et al., 2021	Ross 308	1-42	FF	50-100	-	-	-	-	-	+	-	-
Dabbou et al., 2018	Ross 308	1-35	DF	5-15	+	+	+	-	-	-	-	-
Bellezza Oddon et al., 2021	Ross 308	1-39	FF	5	-	-	-	-	-	-	-	-
Gariglio et al., 2019	R71 L	3-50	FF	3-9	-	-	-	-	-	-	-	-
Schiavone et al., 2019	Ross 308	21-48	FF	50-100	-	-	-	-	-	-	-	-
Gariglio et al., 2021	Canedins R71 L White	3-50	DF	3-9	-	-	-	-	-	-	-	-
Maurer et al., 2016	Lohmann Selected Leghorn	128	DF	50-100	-	-	-	-	-	-	-	-
Kawasaki et al., 2019	Laying hens 168 FF			10 -								

–, no effect of replacing conventional protein sources with BSFL meal; –, decrease as compared to control group; +, increase as compared to control group. ADG, average daily gain; DF, defatted; DFI, daily feed intake; FCR, feed conversion ratio; FF, full fat; FW, final weight.

Table 7. Effect of using BSFL meal as a replacement of conventional protein sources on biochemical and immunological performance in poultry

References	Breed	Age (days)	Type	Level*	Blood metabolites	Hematology	Antioxidant capacity			
							AST	ALT	TAS	GPx MDA
Dabbou et al., 2018	Ross 308	1–35	DF	5–15	No effect on uric acid, creatinine, and cholesterol.	No effect on leukocyte and erythrocyte.	–	–	+	–
Schiavone et al., 2018	Ross 308	21–48	FF	50–100	No effect on uric acid, total protein, triglycerides, cholesterol, creatinine, total protein, GGT and H/L.	No effect on leukocyte and erythrocyte.	–	–	–	–
Bellezza Oddon et al., 2021	Ross 308	1–39	FF	5	No effect on uric acid, total protein, albumin, creatinine, cholesterol, GGT, LDL and H/L.	No effect on leukocyte and erythrocyte.	–	–	–	–
Gariglio et al., 2019	R71 L White	3–50	FF	3–9	No effect on total protein, H/L, GGT and MG. Lowers cholesterol and triglycerides percentages.	No effect on leukocyte, monocytes, lymphocytes, heterophils, basophils, eosinophils, and erythrocyte.	–	–	–	–
Marono et al., 2017	Lohman Brown Classic laying hens	21–45	FF	100	No effect on total protein and H/L. Increases triglycerides, globulin, and cholesterol. Lowers albumin.	No effect on erythrocyte, monocytes, leukocyte, heterophils, basophils and eosinophils.	–	–	–	–
Loponte et al., 2017	<i>Alectoris barbara</i>	7–28	FF	25–50	Lowers BUN and albumin. No effect on total protein, H/L and erythrocyte.	No effect on erythrocyte.	–	–	–	–
Gariglio et al., 2019	Canedins R71 L White	3–50	DF	3–9	No effect on GGT. No effect on MG.	No effect on erythrocyte.	–	–	–	–

*Level of replacement (%) of conventional protein sources with BSFL meal. –, effect on; +, effect on; –, decreased; +, increased compared to corresponding control group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; DF, defatted; FF, full-fat; GGT, gamma-glutamyl transferase; GPx, glutathione peroxidase; H/L, heterophil to lymphocyte ratio; HDL, high-density lipoproteins; LDL, low-density lipoproteins; MG, methylglyoxal; TAS, total antioxidant status.

Table 8. Effect of using BSFL meal as a replacement of conventional protein sources on meat composition and quality in poultry

References	Breed	Age (days)	Type	Level [^]	Performance
Cullere et al., 2019	Ross 708	21–48	FF	50–100	No change in lipid, protein, moisture, and meat ash No change in TBARS and cholesterol. No change in cooking and thawing loss, pH, L*, a*, b*. Increases the n-6/n-3 ratio and total SFA. Lowers PUFA, n-3 PUFA and MUFA.
Murawska et al., 2021	Ross 308	1–42	FF	50–100	No change in aroma strength Lowers cooking losses. Increases pH, a* and b*
Moula et al., 2018	Ardennaise	30–80	FF	2	No change in protein contents of meat in control 24% and BSFL meal 26% No change in $\omega 6/\omega 3$ ratio.
Cullere et al., 2018	Quails	10–28	FF	10–15	Increases C20: 4 ω 6 and total FA in control 33.8% and BSFL meal 36.7%. Increases tyrosine threonine, aspartic acid, alanine, serine and glutamic acid.. Increases the n-6/n-3 ratio and SFA (C10:0, C12:0, C14:0, C16:0 and C20:0). Lowers PUFA, mostly n-3.
Schiavone et al., 2019	Ross 308	1–35	FF	5–15	Increases a*, FA/PUFA ratio and protein. Lowers moisture and b*.
Gariglio et al., 2021	Canedins R71 L White	3–50	DF	3–9	No change in color of thigh and breast muscles as well as pH. Increases α -linolenic acid and C12:0, C14:0. Lowers the ratio of $\sum n-6/\sum n-3$, decreases linearly C18:3 n-3

[^] Level of replacement (%) of conventional protein sources with BSFL meal. a*, red/green value; n-6/n-3, PUFA n-6/PUFA n-3 ratio; b*, blue/yellow value; DF, defatted; FF, full-fat; MUFA, monounsaturated fatty acids; TBARS, thiobarbituric acid reactive substances; PUFA, polyunsaturated fatty acids.

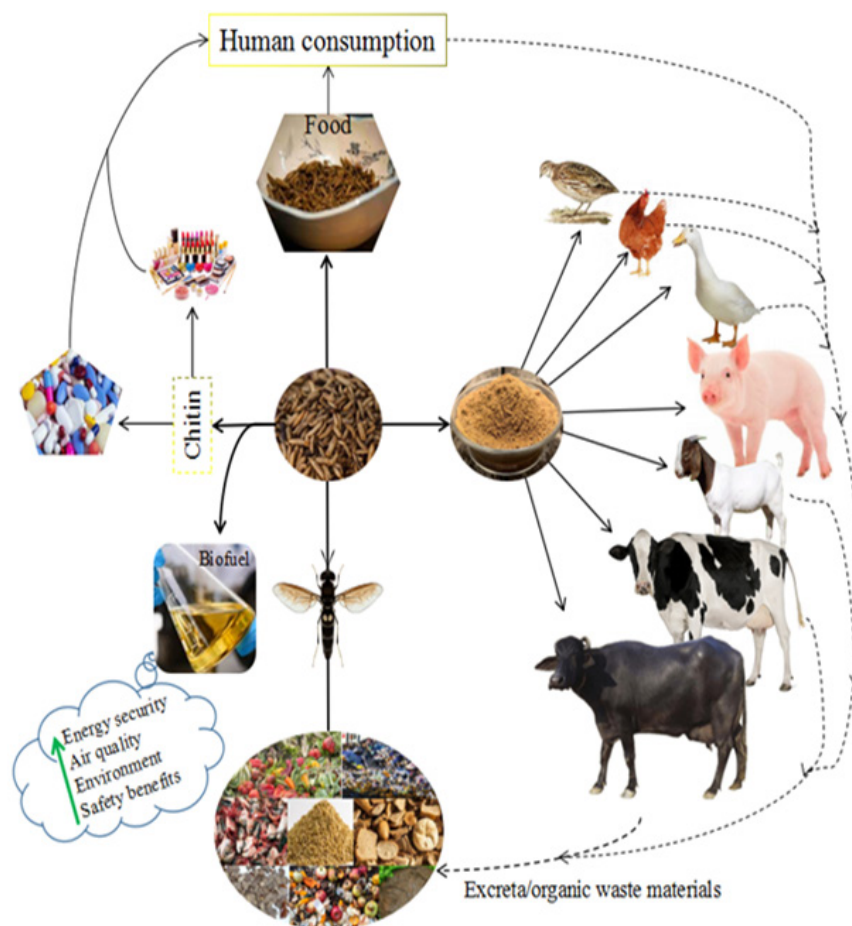


Figure 4. The overall mechanism of using BSFL is to convert organic waste into valuable products. BSFL meals produced from waste could be used as a protein ingredient for animal feed, as protein by humans, as a source of chitin for pharmaceutical companies, or as a source of biofuel. In addition, it is environmentally friendly as it removes organic waste from the soil, which otherwise may pollute the environment

BSFL as a source of chitin

Beyond its nutritional benefits for livestock, BSFL offers a valuable source of chitin, a versatile biopolymer with applications in medicine, biotechnology, and cosmetics (Nagdalian, 2018). Chitin is a structural element found in the cuticle or exoskeleton of the BSFL and is primarily composed of acetyl glucosamine sugar moieties (Joly and Nikiema, 2019). An insect's exoskeleton comprises chitin mixed with proteins, lipids, and other substances.

Chitin's nitrogen level of 6.90% makes it more attractive than cellulose, which has a nitrogen content of 1.25%. In insects, the cuticle is made up of chitin embedded in a matrix of lipids, proteins, and other substances. Chitin is more appealing compared to synthetically substituted cellulose (1.25%) because of its high nitrogen content (6.90%) (Diener et al., 2011 b). Extracting chitin from BSF prepupae has not been economically feasible. Crab and shrimp shells are now the primary industrial sources of chitin. However, it is uncertain if black soldier fly larvae additions may be a suitable alternative (Diener et al., 2011 b). Further research on efficient extraction methods could unlock the full economic potential of BSF as a complete resource and maximize its value across

agriculture and industrial sectors. Figure 4 shows the overall benefits of using BSFL in different fields of life, including agricultural, pharmaceutical, and biochemical industries. In addition, it is also environment friendly as it reduces the amount of organic waste, which otherwise requires huge areas for disposal or may pollute the environment.

Challenges of using BSFL

Security and legal issues

The legislative requirements about food safety are now the principal barriers to the widespread use of BSFL meal in animal feed (Wang, 2017). Restrictive European legislation surrounding the use of insects in animal feed continues to be a significant obstacle to the development of insect production units, despite significant funding for BSFL research (Rehman, 2023). Global food standards set up by the FAO of the United Nations create secure environments for BSFL to produce insects for pet animal nutrition and breeding (Valdés, 2022). This means that they "should not be protected or classified as invasive alien species; they should not be harmful or have other adverse effects on plant, animal, or human health." Additionally, they impose limitations on the substrates that

make up the nutritional supply for BSFL: the substrate must comprise “items of non-animal origin” or a specific group of animal products, such as fish meal, refined lipids, non-ruminant animal blood and gelatin, milk, eggs, honey, etc. Explicit restrictions include meat, manure, “catering waste,” and “other waste” (Žuk-Golaszewska, 2022). Even though the US Food and Drug Administration (FDA) and the American Association of Feed Control Officials (AAFCO) have a formal memorandum of understanding of all animal feed rules, animal feed is not labeled explicitly as food or offered for sale as a novelty snack for people (Veldkamp, 2022; Žuk-Golaszewska, 2022). With the increasing research and potential regulatory reforms, BSFL will probably revolutionize the feed and biomaterial industry.

Consumer acceptance

It is vital to consider how well-liked BSFL-fed animal meat products are with consumers. Insects are frequently a helpful dietary supplement, particularly for poultry and fish. 61% of the participants favored feeding insects to animals (Ribeiro, 2022). Similar observations were published, indicating including insects in fish feed was the most widely agreed upon (Sogari, 2022). This demonstrates that consumers’ perceptions of meat products from insect-fed animals need to be improved and that they still have some remaining questions.

There are observations on humans’ direct intake of BSFL. The Kadazan Dusun tribes in the Malaysian province of Sabah on the island of Borneo consume more than 60 different species of insects. One is BSFL, consumed uncooked with tape, a locally produced fermented beverage (Chung, 2022). Additionally, consumers may not want to eat BSFL directly because of the significant association between dietary saturated fatty acids and the risk of coronary heart disease (CHD) (Purba, 2020). Consumer acceptance remains the hurdle, even though these larvae produce certain antimicrobial peptides that protect themselves against microbes. Further work is required to address consumer preference and awareness about BSFL for animal feed and human food.

Production and economics

There currently needs to be a generally recognized technique for cultivating and handling BSFL. Due to the production equipment’s small size, low yield, and restricted efficiency, there is only a minimal amount of BSFL available for innovators and customers to use in small quantities for scientific study feed (Olutegbe, 2022). The time for mass production has yet to arrive. As a result, BSFL is not frequently employed in the diets of chickens or pigs because its availability and cost are not competitive with those of soybean meal and fish meal (Rehman, 2023). However, addressing production limitations through technological advancements can unlock BSFL’s potential as a sustainable and cost-effective protein source for livestock feed.

Conclusions

Using black soldier flies (BSF) to process organic waste gives benefits such as rapid production time and a high yield at a low expense. BSF technique can convert organic matter into valuable products, including BSF meal, BSF biodiesel, and chitin, with minimal transportation and maintenance needs, leading to substantial financial benefits. This project will explore the advantages of utilizing waste streams as ingredients for BSFL substrate instead of costly and readily available raw materials like wheat bran and maize/cornmeal. By employing such practical techniques, we can convert cheap sources like BSFL oil and protein powder into valuable feed components suitable for many animal feed sectors. In addition, the high protein content of BSFL meal shows significant potential as a protein source in poultry diets. BSFL can replace fish meal or soybean meal as a feed protein source. The establishment of relevant rules has lagged due to the BSFL’s limited application in animal feed. To encourage the manufacture of BSFL and drive down costs, it is required to create standardized production and processing procedures and to strengthen pertinent laws. Another difficulty is getting people to accept meat from animals fed insects. However, BSFL is acknowledged as an efficient waste decomposer, sustainable protein, fertilizer, and biodiesel source, but BSFL adaptation still requires advancement in production techniques, regulatory framework, and consumer acceptance.

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