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Exploring optimum production conditions and nutritional quality of black soldier fly larvae using local bio-wastes for potential use as fish feed in southwest Ethiopia

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Declaration

I, Mehamed Abduraman Fogi, declare that the MSc research entitled ‘Exploring optimum production conditions and nutritional quality of black soldier fly larvae using local bio-wastes for potential use as fish feed in southwest Ethiopia”is original and has been carried out by myself under the supervision of Dr. Mulugeta Wakjira and Dr. Tokuma Negisho. To the best of my knowledge, I confirm that this work has not been submitted for any other degree or qualification at any other university. All sources used in this research have been acknowledged and referenced appropriately. I also declare that any data, figures, or images included in this research are original or have been appropriately attributed to the original source.

Mehamed Abduraman Fogi   Sign----------------------------Date-------------------------

Abstract

Lack of good-quality and sustainable fish feed is one of the bottlenecks for aquaculture development in Ethiopia. Therefore, the study aimed to explore the optimal production conditions in terms of substrate type, feeding rate, feeding frequency, days for maturity, and nutritional quality of black soldier fly (BSF) larvae using local bio-wastes as potential fish feed in southwest Ethiopia. The study involved two trials. The first experiment assessed the effects of local feed ingredients (poultry manure, brewery waste, and rumen liquor) at different feeding rates (100, 150, 200, and 250 g feed/g larvae/day) on BSF larvae biomass production, growth rate, bioconversion rate, waste reduction efficiency, and days for maturity. The amount of substrate added, total weight, dry weight, initial and final larval biomass and maturation time was recorded. Then the recorded data was statistically analyzed using a two-way ANOVA to establish statistical significance among the groups. Brewery waste was found to be the most effective feed ingredient at 100 g feed/g larvae/day feeding rates, which resulted in the highest larval biomass production (12.2 ± 1.03 g), bioconversion rates of 5.19% to 7.89%, and substrate reduction rates of 40.59% to 46.75%. The second experiment examined the mixed feedstocks with varied feeding regimes (daily, midweek, weekly, and lump sum). The best performance was observed on a mixture of poultry manure (Pm) and brewery waste (Bw) with equal proportions (50:50) applied at lump sum feeding regimes. It produced the highest larval biomass (11.05 g), bioconversion rates of 15.48% to 22.78%, and waste reduction rates of 30.92% to 36.54%. The proximate composition of larvae also varied according to the feed ingredient and feeding strategies; the maximum crude protein (CP) content of 46.5% was shown on larvae reared on poultry manure and brewery waste mixed in equal proportions (50:50), whereas the lowest CP content of 39.7% was observed on larvae reared on rumen liquor. The present study paves the way for a more sustainable and localized approach to fish feed production for aquaculture development in Ethiopia, fostering economic growth, environmental stewardship, and improved food security.

Keywords: Aqua feed; Bio-wastes; Black soldier fly larvae; Growth performance; Nutritional value;

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Acronym

DM	Dry Matter
BSFL	Black Soldier Fly Larvae
FAO	Food and Agricultural Organization
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
MC	Moisture content
CP	Crude protein

1. Introduction

1.1. Background of the Study

The global human population is expected to rise to about 9 billion by the year 2050, possibly accompanied by a 70% increase in the demand for animal proteins (FAO, 2011). Population growth leads to a global increase in food consumption patterns, changes in lifestyles, and food preferences (van Huis, 2013). Africa's abundant natural resources, including its vast amount of land, present fantastic opportunities for the growth of aquaculture (Brumes et al., 2008). However, the production of aquaculture in Africa is only marginal, primarily because the production systems are inefficient and there is a shortage of high-quality fish feed (Lazard et al., 2010). Due to the associated economic, environmental, and production issues, it is in jeopardy that traditional feeds like fish meal and soybeans are used as sources of protein in fish feed.

Currently, black soldier fly rearing is done on a small scale in east African countries like Kenya, Uganda, and Tanzania. In Ethiopia, there is limited information available on the existence, status, abundance, and distribution of BSF for use as a potential alternative sustainable protein-rich diet for poultry and fish feeds (Edea et al., 2022).The larvae may typically reduce waste by up to 60%, and the leftover residue provides a good and safer fertilizer to utilize for plant growth, especially when the larvae's exuviate is added to the fertilizer (Lee et al., 2013; Newton et al., 2005).

There are two major problems with using fish meal for animal feed, including aquaculture practices. The use of the black soldier fly (BSF) as a bioreactor to convert local bio waste into nutritionally improved fish feeds and other useful products represents a potential option. This bioconversion of waste into aqua feed represents a viable and sustainable solution to the challenges of fish feed in order to promote aquaculture development and address environmental issues. The BSF-based aqua feed also has the benefit of improving fish health via its antibiotic nature. Moreover, BSF biodegradation has also been shown to produce very rich organic manure (compost) as a byproduct, which can be used to address the loss of soil fertility, which is one of the challenges facing agricultural productivity and affecting food and nutritional security.

Optimization of growth at the larval feeding stages for BSFL generation, directly influences pupa development, adult lifetime, and the fecundity and viability of eggs produced (Holmes et al., 2012). This is significant since it has been demonstrated that larval growth was affected by diet content, food rationing, and feeding frequency. Because consumption and utilization have been discovered to vary, the ideal levels of these parameters must be determined by strain- and substrate-by-substratum (Kim et al., 2011).

Despite the fact that many research projects have been done on BSF, the impact of different substrate types on the nutritional quality of black soldier flay biomass, as well as the feeding strategy on local substrates, which includes determining optimal feed rates, regimes, and substrate mixing, has not been thoroughly investigated. In addition, the amount of bio-waste used in earlier projects and the geographic area they covered were constrained. Therefore, this research projects used locally abundant wastes, such as poultry manure, rumen liquor and brewery waste, as experimental substrates to encourage the quality and quantity of BSF larval productions.

1.2. Statement of the Problem

Africa's abundance of land and other natural resources presents fantastic opportunities for the advancement of aquaculture (Brummett et al., 2008). Nevertheless, due to production system inefficiencies brought on by a lack of high-quality fish feed, aquaculture production in Africa is only marginal (Lazard et al., 2010).

The use of conventional feeds like fish meal and soybean as sources of protein in fish feed is in danger due to the associated economic, environmental, and production difficulties. The twin issues of waste management and protein deficiency may be solved by BSFL farming, which uses organic waste as a substrate. For BSFL to serve effectively in the dual roles of biomass production and attendant waste reduction, the establishment of a production system with a feeding strategy that specifies important substrate factors that affect BSFL feeding behavior, growth, and development is necessary (Devic and Maquart, 2015). The requirement to choose appropriate production substrates for both product quality and quantity arises from the fact that not all organic materials are suitable for BSF production in captive operations (Zheng et al., 20). In addition to substrates, management techniques like environmental factors and feeding strategy have an impact on BSFL productivity in captive operations. These activities' regulation moves the focus from only raising BSF to facilitating efficient consumption and, consequently, waste reduction.

Although there are multiple BSF research projects, the impact of different substrate types on the nutritional quality of insect biomass as well as the feeding strategy on local substrates, which includes determining optimal feed rates, regimes, and substrate mixing, has not been adequately examined. Furthermore, earlier projects had a limited geographic reach in order to fulfill their objectives. This study was aimed at improving the production circumstances and feed quality of black soldier fly larvae biomass derived from locally available bio waste such as poultry manure, rumen liquor, and brewery waste for use in aquaculture

1.3. Research Questions

The study tried to answer the following major research questions:

1. How do different kinds of bio waste at different feeding rates affect biomass, growth performance (weight gain), bioconversion rate, substrate reduction rate, and maturation days of the BSF larvae?

2. What is the best combination of bio wastes at different feeding regimes for increased biomass, growth, bioconversion, substrate reduction, and maturation days?

3. How does the proximate composition of BSF larvae vary in response to varied diet ingredients and feeding strategies?

1.4. Objectives

1.4.1. General Objectives

The study aimed to evaluate the optimal production conditions and nutritional quality of black soldier fly larvae from locally available bio wastes for possible use as fish feed for aquaculture development in southwest Ethiopia.

1.4.2. Specific Objectives

- To determine potential of locally available bio wastes and optimum feeding rate for better biomass, growth performance, bioconversion rate, substrate reduction rate of BSF larvae, and maturation days.
- To determine the optimum combination levels of ingredient substrates and feeding regimes for better BSF larvae growth performance, bioconversion rate, and substrate reduction rate.
- To evaluate the proximate composition of BSF larvae reared on different feed ingredients and feeding strategies

1.5. Significance of the Study

Edible insect production, such as BSFL, produces fewer greenhouse gases and hence aids in minimizing climate change and its consequences (Oonincx et al., 2010). The study's contributions to black soldier fly sustainable farming through identification of feeding substrates and optimization of production through a feeding strategy help to reduce reliance on wild larvae gathered and halt biodiversity loss in the terrestrial environment.

The development of a feeding strategy and techniques for BSFL is a direct contribution to sustainable consumption and production patterns through sustainable management and effective use of natural resources through waste prevention, reduction, recycling, and reuse. This is related to food security, better nutrition, and the development of sustainable agriculture, all of which strive to create a circular economy. The determination of the optimum feeding rate on the substrates used for BSFL growth is critical from both an economic and biological standpoint and also contributes to a feeding strategy that can improve growth performance, survival, food conversion ratios, size variation, and waste management problems, as well as minimize food waste and money spent on feed and thus increase production efficiency.

2. Review of Related Literature

2.1. Aquaculture production for food security and nutrition

Global fisheries and aquaculture production is at a record high, and the sector will play an increasingly important role in providing food and nutrition in the future. In 2020, global aquaculture production reached a record 122.6 million metric tons, including 87.5 million metric tons of aquatic animals worth USD 264.8 billion and 35.1 million metric tons of algae worth USD 16.5 billion. Inland aquaculture produced approximately 54.4 million metric tons, whereas marine and coastal aquaculture produced 68.1 million metric tons (FAO, 2022). Aquaculture's contribution to worldwide aquatic animal production reached a record 49.2 percent in 2020 (FAO, 2022).

Aquaculture continued to grow in all regions in 2020, with the exception of Africa, and was primarily fueled by expansion in Chile, China, and Norway, the top producers in each region. Egypt and Nigeria, the continent's two largest producers, had a decline, while the rest of Africa saw growth of 14.5% in 2019. Asia continued to rule the aquaculture industry, producing more than 90% of the total (FAO, 2022). Ethiopia is a country in the Horn of Africa that is rich in fish resources, water resources, and environmental resources. However, it is ranked last among aquaculture producers despite having over 20 natural lakes, 12 massive river basins, over 75 wetlands, and 15 reservoirs (Abebe, 2016).

Fisheries are critical to the global food economy. Food security is more than just food production. It is described as the physical and economic availability of sufficient, safe, and nutritious food to meet dietary requirements (Gareth, 2001). Fish play a key role in food security. It is a primary source of protein and essential nutrients and provides income and livelihoods for numerous communities across the world, including small-scale fisheries. The amount intended for human use (excluding algae) was 20.2 kg per capita, which was more than double the 1960s average of 9.9 kg per capita. The primary sector employed an estimated 58.5 million people. It is estimated that 600 million livelihoods are dependent on fisheries and aquaculture, including subsistence and secondary sector workers and their families (FAO, 2022).

Fisheries are an important component of food security, particularly for many poor people in developing countries. They are utilized in food-insecure, low-income countries. Fish and fishery products, according to the Food and Agricultural Organization (FAO, 2014), play an important role in food security and providing the nutritional demands of the human population in both developing and developed countries. Fish, in particular, promotes nutritional security by providing high-quality protein as well as an important source of micronutrients such as vitamins, minerals, and polyunsaturated omega-3 fatty acids (FAO, 2012). Omega-3 fatty acids are essential nutrients for appropriate newborn brain and eye development, as well as being protective against a variety of human disorders such as cardiovascular disease, lupus, depression, and other mental illnesses (Alawode, 2018).

2.2. Aqua feed as a Major Factor in Aquaculture Development

The availability of inexpensive protein sources during the formulation of aqua feed is strongly tied to the performance of the aquaculture sector (FAO, 2018). Due to its many advantages, such as its well-balanced amino acid composition, improved digestibility, and palatability, fish meal (FM) is one of the principal protein sources. It is also crucial for enhancing the uptake, digestion, and absorption of other nutrients in fish diets (Miles and Chapman, 2006). The risk posed by using FM as the primary source of protein in compounded aqua feeds, however, may drive feed producers to rely on it. Feed formulators therefore look for replacement feedstuffs that can replace FMs without affecting fish performance (Daniel, 2018).

In the production of aquaculture, aqua feeds are crucial. Around 50% of the variable manufacturing expenses are borne by them (Rana et al., 2009). The alternative protein sources are either of plant or animal origin. Plant protein sources include soybean meal, cottonseed meal, and ground nut/sunflower oil cake meal (Kestemont et al., 2007).Due to their low cost and ease of obtaining, plant protein sources, particularly soybeans, have been extensively researched as fish protein sources (Gatlin et al., 2007). However, because of concerns regarding the protein's quality and amino acid composition, palatability, phosphorus bioavailability, digestibility, and the presence of anti-nutritional agents, the use of plant protein in animal feed has been restricted (Gatlin et al., 2007; FAO, 2012). It has been shown that plant protein sources, particularly in carnivorous fish, cause morphological alterations in fish digestive tracts (Krogdahl et al. 2003).

The lamina propria of the mucosal folds widen and shorten, enteritis occurs, inflammatory cells are infiltrated into the lamina propria, and the absorptive cells in the intestinal mucosa lose their supranuclear vacuolization. The by-products of terrestrial animals, such as meat meal, blood meal, meat and bone meal, hydrolyzed feather meal, poultry by-product, and liver meal, are occasionally mine rally imbalanced and low in certain amino acids (Tacon, 2020). Due to protein degradation, byproducts of the fishing industry, like fish silage, have significant quantities of free amino acids (Hertrampf & Piedad-Pascual, 2000).

As a new source of protein for animal feed, insects, particularly fly larvae, have recently gained popularity. Meals made from fly larvae may one day serve as a source of protein for fish. Larvae meals have been suggested as a potential substitute due to their high nutritional value and low cost of production when compared to other protein sources (Ogunji et al., 2006). Men typically don't consume larval meals directly. Insect larvae can be raised on a variety of waste materials, including animal dung and food scraps, producing a nutrient-rich supply while also reducing and transforming those organic wastes (Henry et al., 2015).

2.3. Insect Meal as a Viable Option for Aqua Feed

A more sustainable source of protein for animal feed is provided by insects that can be produced from organic waste. Use of farmable edible insects as feed ingredients is associated with certain advantages: they are rich in proteins, fat, energy, vitamins, and minerals; have higher feed conversion efficiency compared to livestock and therefore use less feed; require less land than crop production; have great acceptance from poultry and fish as part of their natural diet; and are mostly omnivorous and therefore grow on different substrates (van Huis et al., 2013). However, despite the tremendous potential to be used as a feed item for many livestock animals, they are currently not widely used, perhaps due to inadequate knowledge about their potential (Devic, 2016). Insects, particularly fly larvae, have recently become a new source of protein for animal feed. Meals made from fly larvae may one day serve as a source of protein for fish. Larvae meals have been suggested as a potential substitute due to their high nutritional value and low cost of production when compared to other protein sources (Ogunji et al., 2006; Aniebo et al., 2009). Men typically don't consume larval meals directly (Teotia& Miller, 1974). Insect larvae can be raised on a variety of waste materials, including animal dung and food scraps, producing a nutrient-rich supply while also reducing and transforming those organic wastes (Aniebo et al., 2009).

Due to the necessity to find a substitute for fishmeal in animal feeds, the black soldier fly (BSF) has been employed as a fish feed; however, research has only been conducted with rainbow trout, channel catfish, Ictalurus punctatus, and blue tilapia. Without causing any negative effects, the larvae can replace up to 25% of the fishmeal used, but no research has been done on the possibility of using BSF larvae as a full replacement. Since fish naturally eat insects, there is a lot of potential for using insects as fish food (Rumpold & Schlüter, 2013). According to Henry et al. (2015), the majority of insects and their larvae have a well-balanced amino acid profile and a high lipid, mineral, and vitamin content.

In addition, most organic waste streams can be used to raise insects, which can turn the waste into a high-quality feedstock while lowering its inherent contamination potential (Barroso et al., 2014). In addition, compared to animals, insect farming produces fewer greenhouse gases (Oonincx et al., 2010). Generally, insect species can be farmed organically to reduce environmental contamination and convert waste into high-protein feed, possibly substituting fish meal in aqua feed. This sustainable technology uses less area and water than crops and has a lower carbon footprint, making it a feasible option for aquafeed production (Stejska et al., 2023) Insects are efficient food converters because they can maintain high body temperatures without consuming energy (Nijdam et al., 2012). However, not all insect species are suitable as feed elements in aquaculture. Only a few species are suitable for large-scale production, depending on their protein, amino acid profile, fat, mineral content, and raw material availability (Laura et al., 2018). The most common insects are the black soldier fly, housefly, and yellow mealworm. Black soldier fly larvae can grow on byproducts such as digested food, vegetables, and restaurant leftovers, making them a desirable protein element for industrial aqua feed production (Spranghers et al., 2017).

2.4. The Mounting Challenge of Global Bio-Waste

Biowaste, which includes everything from food scraps to yard clippings, makes up a significant portion of worldwide solid waste. While seemingly harmless, improper bio-waste disposal causes a plethora of problems. From environmental effects to public health concerns, efficiently dealing with bio-waste necessitates a multifaceted approach (Vea & Thomsen 2018). One of the most pressing concerns regarding biowaste is its contribution to landfill issues. When put in landfills, biowaste decomposes anaerobically, releasing methane, a potent greenhouse gas with a substantially higher warming capacity than carbon dioxide (EPA, 2003). Furthermore, congested landfills consume valuable space and might contaminate surrounding water sources with leachate, a hazardous liquid produced by decomposing waste (Kaza et al. 2018).Improper bio-waste disposal causes public health issues. When bio-waste is mixed with regular garbage, it attracts pests such as rodents and insects, which can spread diseases (WHO, 2022). Furthermore, in some areas, bio-waste is burned in open pits, releasing dangerous air pollutants that cause respiratory problems (Weber et al., 2019). Open dumping and burning have been superseded as the main techniques for managing solid waste by initiatives that focus on resource reuse, recycling, and consumption reduction (Mutafela, 2015). Organic waste is rarely recycled or reused (Henry et al., 2006; Hoornweg and Bhada-Tata, 2012).

This is especially true in underdeveloped nations where a lack of resources makes it difficult to incinerate garbage by installing effective machinery and technologies that can turn organic waste into energy (UN-HABITAT, 2010). Consequently, anaerobic digestion and composting are two great methods for turning biowaste into valuable resources. Anaerobic digestion produces biogas, a fuel that burns cleanly, while composting transforms organic matter into nutrient-rich fertilizer (Vea & Thomsen, 2018). Thus, innovative approaches to waste management are required, including the utilization of the bioconversion process, which can even add value to the waste (Mutafela, 2015). Black soldier larvae are voracious eaters that flourish on a wide range of organic resources, such as water hyacinth biomass, animal feces, brewery waste, municipal trash, and kitchen and market waste. These materials are selected for their accessibility, affordability, non-human use, and environmental sanitation.

2.4. Distribution and Entomology of the Black Soldier Fly

Hermetiaillucens (BSF) is a true fly (Diptera) in the Stratiomyidae family. The bug is native to the American continent's warm tropical and temperate zones. (Newton et al., 2005). Climate change and human activities have accelerated its spread to other continents (Leek, 2017). They are generally regarded as beneficial insects and non-pests. It is one of the potential protein source feed ingredients due to its ability to convert large amounts of organic waste (1.3 billion tons per year) into protein-rich biomass (Veldkampet et al., 2012). Black soldier fly larvae are naturally found in poultry, pig, and cattle manure, but they can also be grown on organic wastes such as coffee bean pulp, vegetables, catsup, carrion, and fish offal. Adult BSF only looks for a mate, breeds, and lays about 500 eggs in crevices near composting waste and measures 15-20 mm in length (Diener et al., 2011; Hardouin et al., 2003). The female's abdomen is mostly black, with a reddish apex and two translucent spots on the second abdominal segment. The adult fly lacks mouth parts and does not feed during its brief lifespan. They do not bite or sting, only feed as larvae, and are unrelated to disease transmission. Adult flies can be identified by their long antennae (Gennard, 2012).

2.5. Life Cycle of the Black Soldier Fly and Reproductions

The BSF has five life stages: egg, larvae, prepupae, pupae, and adult. BSF has an estimated life cycle of 40 days, but this varies depending on the environmental conditions and nutrition provided (Alvarez, 2012). Under ideal conditions of 20°C to 30°C, eggs are typically creamy yellow in color and need 4 days to incubate and hatch (Newton, 2015).

BSF larvae have a dull, yellowish hue immediately after hatching and try to hide away from light due to their photophobic nature. Larvae are voracious consumers of organic stuff and grow quickly. The larvae spend the majority of their lives grazing on food and manure wastes, which they quickly convert into fat, protein, and calcium. These nutrients are used by larvae to develop into pupae and then into adults (Newton et al., 2005).Additionally there was a figure that give brief explanation in the prepared appendix 1

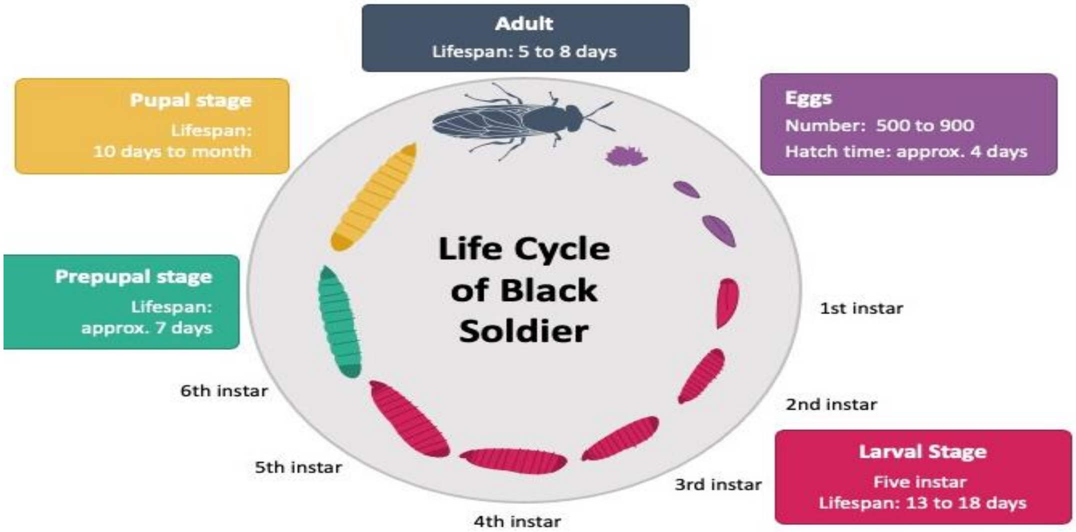

Fig. 1.Life cycle of black soldier flay. https://www.google.com/imgres?

With optimum temperatures, larvae will reach full size (20 to 25mm) in about 4 weeks. When the BSF larvae stop feeding, they become dark, and their skin becomes harder. This is the pre-pupa stage; at this point, they start searching for a dry and dark place other than a feed source to pupate and transform into adults (Newton, 2015; Burtle et al., 2012). Pupation can last 5 to 7 days, depending on temperature and ambient humidity. Adults emerge after 10–14 days at 27–36 C from their pupae cases. The adults do not feed but rely on fats stored from the larval stage for their life activity. (Myers et al., 2008; Newton, 2015). Adults live and mate two days after emerging from the pupal stage (Myers et al., 2008). Female oviposit into dry cracks and crevices near larval habitat (Newton et al. 2005) two days after copulation. A temperature of 25°C–35°C (Newton, 2015) and ambient light play a vital role in initiating mating for adult flies, as it is found in the studies that mating levels of adults were highest under natural sunlight. Furman et al. (1959) stated that BSF mating begins in the air with aerial questing after stimulation by light (Alvarez, 2012). The adult flies are photophilic and require strong daylight spectra as well as temperatures between 25°C and 35°C to encourage mating to occur.

2.6. Contributions of Black Soldier Fly Larvae to the Environment

2.6.1. As live feed for livestock and fish

As the world's population grows, so will the demand for human and animal feed (FAO, 2022). To fulfill the increased need, alternative food and feed production technologies must be developed. Insects are increasingly regarded as a new source of protein for human and animal consumption (Huis et al., 2013). The protein content of insects is comparable to that of fishmeal and soybean meal, particularly in terms of lipids, amino acids, minerals, and vitamins (Henry et al., 2015). Dependence on fish meal in fish diet production has impacted aquaculture revenues (Olsen & Hasan, 2012) and environmental sustainability (Collavo et al., 2015).

The larvae of insects that are very rich in protein and dietary fat, like termites, houseflies (Musca domestica L. (Diptera: Muscidae), and black soldier flies (Hermetia illucens L. (Diptera: Stratiomyidae), can be used in animal feed to lessen the use of unsustainable protein sources, like fishmeal and soybean (Bosch et al., 2020; Yang & Tomberlin, 2020). Because Hermetia illucens larvae can be raised on a variety of wastes, including food waste, cattle manure, and agro-industrial waste, they are very well-liked (Nguyen et al., 2015; Chia et al., 2018; Danieli et al., 2019). For monogastric species like fish, poultry, and pigs, fresh or dried larvae can be fed as a source of protein (Tomberlin, 2019; Wang & Shelomi, 2017; Dörper & Dicke, 2021).

The larvae and prepupae of black soldier fly have a high protein and fat content (Oonincx et al., 2015) that can be used to support the growth a lot of livestock, such as blue tilapia fish and pigs (Dierenfeld et al., 2008). The environmental impact of animal husbandry could decline significantly if black soldier fly larvae are used in order to eliminate livestock manure and be reused as livestock feed. Organic matter as a growing substrate greatly affects the development of the black soldier fly and various biological traits (van Huis et al., 2013).

The BSF larvae can, depending on the feeding medium, contain high concentrations of lipids (>30% of dry weight, dw) and protein (around 40% of dw) (Hilaire et al., 2007; Diener et al., 2009). Recently, interest in using BSF larvae as a raw material for animal feed has increased, mainly due to its potential as a sustainable source of high-quality protein (Kroeckel et al., 2012; Vandkamp et al., 2012).

2.6.2. Biomass conversion

A wide range of organic materials, such as agricultural waste, kitchen leftovers, and animal dung, can be consumed by BSFL. Certain waste materials, like rice straws, are known to be challenging to valorize due to their high lignocellulosic matter content, making them unsuitable for use as animal feed (Zheng et al., 2012; Manurung et al., 2016). All of these wastes indicate decreased revenue in terms of lost nutrients and disposal costs, some of which are potentially biohazardous contaminants and/or draw pest flies that spread disease. Manure is already successfully managed with the help of wild BSFL, which lowers odor and pest fly populations (Sheppard et al., 2002). Bioconversion of food wastes by black soldier larvae (BSFL) is a promising solution for the management and valorization of organic waste streams (Lalander et al., 2019; Gold et al., 2020). The BSFL can feed on a wide range of organic substrates, including food waste, processing residues, and human and animal fecal wastes, to efficiently convert organic matter (OM) into a high-value source of protein and fat biomass that provides sustainable solutions for both organic waste management and food security (Lalander et al., 2019; Koné, 2020). Several researchers have shown that BSFL is effective at reducing animal manure and organic waste materials by converting them into a protein- and fat-rich biomass suitable for various purposes, including animal feeding, biodiesel, and chitin production (van Huis et al., 2013). The larva of the black soldier fly (BSF) is a scavenger commonly used to accelerate the composting of organic material. BSF larvae can efficiently utilize organic resources, like fruit, vegetable, and meat waste (Čičková et al., 2015). Kim et al. (2011) reported that BSF was able to consume and digest raw organic waste materials (manure, kitchen waste, abattoir waste, blood, and offal's) more rapidly and resourcefully than the house fly (*Musca domestica*).

2.6.3 Odor and pollution reduction

Odor and pollution reduction are other benefits derived from BSF. This is accomplished by their abundant densities on waste material combined with their avid appetite, causing the waste material to be processed at a fast rate while the larvae are processing this waste; they aerate and dry the material, suppressing bacterial growth (Edea et al., 2022). The larvae modify the microflora of manure, potentially reducing harmful bacteria such as Escherichia coli 0157:H7 and Salmonella enterica (van Huis et al., 2013). The BSF larvae reduce the nutrient concentration and the amount of manure residue, leading to a reduction in the amount of pollution, possibly by 50–60% or more (Newton et al., 2005), and causing it to be less favorable to the house fly larvae. The combination of all these characteristics causes a reduction in odors and pollution (Diener et al., 2011).

2.6.4. Housefly control

The common housefly (M. domestica) tends to come into more contact with humans for a number of reasons. The common housefly feeds throughout its life due to its physiology of having functional feeding parts. This causes the fly to always be on the lookout for edible organic matter, such as human food, making the interaction between the fly and humans more common (Edea et al., 2022). The BSF's physiological traits of having no functional feeding parts cause it to have no attraction to homes, consequently reducing any pest-like behavior and living its life apart from humans (Barry, 2004). However, the BSF has a strong ability to reduce the number of house flies by preventing the house fly from ovipositing (the act of depositing eggs). The reduction of house flies will be a large benefit as they are prominent disease vectors, adding to the importance of their population control. The ability of colonization by BSF was reported by Sheppard et al. (1994), who discovered that BSF had the ability to colonize poultry and pig manure, causing a reduction in common housefly populations by 94–100%.

2.6.5. Chitin benefit

Apart from having a desirable (soluble) protein content, insect species also contain high amounts of chitin, which is the main constituent in the insect exoskeleton. Chitin is a non-toxic, biodegradable linear polymer (Edea et al., 2022). Recent studies confirmed that chitin has effects on innate and adaptive immune responses, including the ability to recruit and activate innate immune cells and induce cytokine and chemokine production via a variety of cell surface receptors, including macrophage mannose receptor, toll-like receptor 2 (TLR-2), and Dectin-1 (Lee et al., 2008).

2.7. Nutritional Compositions of the Black Soldier Fly

Because fish eat insects naturally, insects have enormous potential for use as fish food (Henry et al. (2015) discovered that most insects and insect larvae have a well-balanced amino acid profile and are rich in lipids, minerals, and vitamins). Furthermore, the majority of organic waste streams Because fish eat insects naturally, insects have enormous potential for use as fish food (Henry et al. (2015) discovered that most insects and insect larvae have a well-balanced amino acid profile and are rich in lipids, minerals, and vitamins). Furthermore, most organic waste streams can be used to raise insects, converting the waste into a high-quality feedstock while reducing the waste's inherent contamination potential (Barroso et al., 2014). Furthermore, when compared to animals, insect farming produces fewer greenhouse gases (Oonincx et al., 2010). The literature has documented various nutritional values for insects, particularly fly larvae. The BSF larvae may have significant concentrations of lipids (>30% of dry weight, dw) as well as protein (about 40% of dw), depending on the feeding medium (Hilaire et al., 2007; Diener et al., 2009). (Barroso et al., 2014). Furthermore, when compared to animals, insect farming emits fewer greenhouse gases (Oonincx et al., 2010). The literature has documented various nutritional values for insects, particularly fly larvae. Depending on the feeding medium, BSF larvae may have significant lipid (>30% of dry weight, dw) and protein (about 40% of dw) concentrations (Hilaire et al., 2007;

Diener et al.,2009).Black soldier fly (Hermetiaillucens) larvae contain up to 35% extract and 47% crude protein on a dry matter basis, and BSF larvae or pre-pupae are high-quality feedstuffs. (St-Hilaire et al., 2007)According to Makkar et al. (2014), the crude protein content of black soldier fly larvae is 42.1%; however, defatted black soldier fly larvae have 56.9% crude protein, which is roughly equal to soybean meal but slightly less than fish meal. Protein meals with fat concentrations ranging from 3.4 to 38.6% of DM are produced by processing and separating a lipid component from black soldier fly larvae. Fish meal can be replaced more effectively with black soldier fly larvae because they have a better amino acid profile than soybean meal (Tran et al., 2022). But before processing, the oil needs to be extracted from the biomass of black soldier fly larvae (Stamer, 2015). They are heavy in ash, calcium, and phosphorus, and their nutritional status peaks during the pupal stage. The maximum shelf life is achieved at 10 to 16 °C (50 to 60 °F); however, they can be kept at room temperature for several weeks.

3. The Study Area and Methods

3.1. Study Area Description

This experiment took place at Jimma University's Aquaculture Laboratory, Department of Biology. The region was situated at an elevation of 1700 meters above sea level, 350 kilometers southwest of Addis Abeba. The average yearly rainfall and daily temperature in the study area were 123.01 mm and 20.71°C, respectively (Jimma Meteorological Station, 2023).

3.2. Establishing BSF Colony

We collaborated with the researchers at Addis Ababa University (AAU) in the field of insect science who donated the pupae stage of the fly to enable us establish the BSF colony at Jimma University. The pupae were placed in a cage inside a mini-greenhouse to allow them to hatch into adult insects (Figure 2). Following the emergence of adult insects, a mix of different substrates (fruit and vegetable left overs, animal waste, maize bran) and egg tarps (cardboard and wooden traps) were employed as attractants and sites for egg laying by the adult insects. The eggs were then placed on a fine wire mesh above a mixture of substrates (wheat bran) to provide a suitable environment for the larvae to hatch and grow.

Fig 2. Establishment of the adult colony of black soldier fly at Jimma University: (A) Mini-green house for the BSF rearing, (B) Attractants and egg traps within the mini-greenhouse for the adult fly rearing, (C) Pupal stage of the reared BSF, and (d) Adult BSF emerged from the pupa stage.

3.3. Collection of Substrates

Various samples of bio-waste from different sources, such as rumen liquor (an abattoir waste), poultry waste, and spent grains from a brewery, were collected and processed to be used as substrates for the production and experiments involving Black Soldier Fly (BSF). The rumen liquor and poultry waste were obtained from the Jimma Town abattoir center and local chicken-rearing areas in and around Jimma Town. These bio-wastes were then transported to Jimma University for further processing into usable forms for the experiments (Figure 3). The selection of these bio-wastes for the experiment was based on factors such as local availability, sustainability, cost-effectiveness, non-competition with other uses (especially human uses), and the necessity to manage the wastes effectively (Gabriel et al., 2007).

Fig 3.Samples of local bio-wastes collected and processed for the experiments

3.4 Experimental Design

Evaluation of the optimum feeding rate for various ingredient (non-combined) substrate types followed a factorial design with four levels of feeding rates (FR) and three levels of substrate types (ST), in triplicates, with a mid-weekly feeding frequency (Manyara,2018) (Table 1). Each treatment group consisted of 100 larvae with initial average biomass of 6.0 g, 6.16 g and 6.25 g for poultry waste, rumen liquor and brewery waste substrates, respectively. The newly hatched neonates were fed commercial chick mash until they reached the age of 5 days. On the sixth day, the larvae were sieved through a 1.2-mm mesh screen, weighted, and sorted for the experimental rearing substrates until the end of the experiment.

Before starting the experiment, it was ensured that the slight variations among these initial average biomasses of the three treatment groups were statistically not significant (one way ANOVA, p = 0.858666). The detailed statistical analysis is provided in appendix 1. The experiment spanned for 13-17 days.

Table 1.Summary of the experimental design to evaluate optimum feeding rate for various individual (non-combined) substrate types of black soldier fly.

	Feeding Rate (g/Larva/day)			
Substrate	Treatment-1	Treatment-2	Treatment-3	Treatment-4
Poultry waste	100	150	200	250
Rumen liquor	100	150	200	250
Brewery waste	100	150	200	250

Likewise, to assess the ideal feeding regimen for various substrate combination levels, the study utilized a factorial design with four levels of feeding regimes (FR) and three levels of substrate combinations (SC), each in triplicate, at a feeding rate of 100g per larva per day (Table 2). Each treatment consisted of 100 larvae with an initial larval biomass of 3.3g, 2.9g and 2.83g. The experiment was conducted over eighteen days.

Table 2.Summary of the experimental design to evaluate the ideal feeding regime for various Substrate combination levels on poultry manure (Pm), rumen liquor (Rl) and brewery waste (Bw)

	Feeding frequency			
Substrate combination	Treatment-1	Treatment-2	Treatment-3	Treatment-4
50%Pm:50%Rl	Daily	Mid-week	Weekly	Lump sum
50%Pm:50%Bw	Daily	Mid-week	Weekly	Lump sum
75%Pm:25%Rl	Daily	Mid-week	Weekly	Lump sum

3.5. Biological Data Collection

Data was collected every four days, for about 12-16 days, depending on the larvae's development stage. The data for rumen liquor and brewery waste was collected in four rounds, but poultry manure was collected in three rounds due to the rapid maturation rate observed. This data was collected for each ingredient at various feeding rates. Data for the mixed ingredient with different feeding regimes were collected in two rounds at the beginning and end of the experiments, i.e. on the first day and after eighteen days.

In general, biologically recorded data for the studies maximizing BSF production were the initial and final larval biomass (g), amount of substrate provided, total substrate weight, dry weight, moisture, maturation period, and proximate composition of larvae.

3.6. Proximate Analysis

The proximate study of substrate composition and larvae grown on bio wastes was conducted on one gram of dry matter for each variable analyzed. Proximate analysis (carbohydrate, protein, fat vitamins and essential minerals) of the BSF larvae produced from the optimization experiments and organic bio wastes samples were analyzed following standard procedures (AOAC, 1990; Rehbein and Oehlenschläger, 2009) at Jimma University's Laboratory of Agriculture and Veterinary Medicine.

3.6.1. Moisture determinations

The moisture content was ascertained by subjecting 2 grams of the sample to a 24-hour oven drying process at 105°C, in compliance with Official Method 934.01 of the Association of Official Analytical Chemists International (2002). A formula was used to compute the moisture.

$$MC = \frac{(WWS - WDS) \times 100}{WWS}$$

Where, MC = Moisture content, WWS = Weight of wet sample, WDS = Weight of dried sample

3.6.2. Crude Protein determinations

The crude protein in the black soldier fly larvae was measured using the AOAC's (1990) Kjeldahl techniques. The material was digested in a Kjeldahl digestion flask, heated to 370°C for four hours, then cooled, neutralized, and diluted. The solution was then transferred to a 250-mL flask attached to a distiller. The distillation process was stopped when the volume reached 200-250 mL. The nitrogen content was determined by titrating the borate anion produced with 0.1N HCl and calculating the amount of nitrogen using the formula. The blank was treated with all reagents except the sample.

$$\% N = \frac{N\,HCl \times (Vol\,HCl\,TS - Vol\,HCl\,TB) \times 14\,g \times 100}{Gram\ of\ sample\ mole}$$

Where, TS = titrates sample, TB = titrates blank

Crude protein = 6.25 × N

3.6.3. Crude Fat Determination

Crude fat was calculated using the Soxhlet method, which is a semi-continuous solvent extraction method recommended by the AOAC (1990). Two grams of dried and powdered samples were placed in a porous cellulose extraction thimble and coated with fat-free cotton. The thimble was suspended in an extraction chamber above a flask of diethyl ether. The flask was dried, heated, and then evaporated. The solvent was then taken to the condenser and turned into a liquid. The lipid mass was determined gravimetrically and expressed as a percentage. The initial sample's crude fat content was determined.

$$Fat\ content = \frac{Weight\ of\ fat \times 100}{Weight\ of\ sample}$$

3.6.4. Ash Determination

The AOAC technique (1990) was used to determine the amount of ash. In brief, duplicates of 2.50 g of homogenized samples were placed in pre-washed, dried, weighed, and marked crucibles to be heated in a muffle furnace at 550 °C for eight hours.

The samples were then chilled in a desiccator and reweighed. The ash content was computed as follows:

$$\%\ Ash\ (wet\ basis) = \frac{(WAA - TWC) \times 100}{Original\ sample\ weight}$$

Where, WAA = weight after ashing, TWC = tare weight of crucible finally, proximate composition in wet base was computed from dry base using the following formula:

$$\%\ Proximate\ in\ wet = \frac{\%\ \mathrm{PID} \times (100 - \mathrm{MC})}{100}$$

Where, PID = Proximate in dry, MC = Moisture content

3.6.5. Crude Fiber Determination

Crude fiber was determined using an ANKOM fiber analyzer (ANKOM 220, USA) using the filter bag technique. A 0.1 g sample was packed in an ANKOM filter bag F57 and immersed in petroleum ether for 10 minutes to extract the fat. Then it was air-dried at room temperature and placed in a fiber analyzer vessel. A solution of 0.255 N H2SO4 was added, and the samples were allowed to extract for 40 minutes. Afterward, the samples were washed twice with hot water. The samples were then treated with 0.313 NaOH and allowed to extract for 40 minutes. The samples were washed three times with hot water before soaking in acetone for 5 minutes. The samples were dried in an oven at 105°C and ashed in a furnace for 2 hours at 550°C.Crude fibre content was calculated using the formula

$$\text{Crude fiber} = 100 \times \frac{W3-(W1\times C1)}{W2}$$ Where:

W1= Bag tare weight W2= Sample weight

W3= Weight of organic matter (loss of weight on ignition of bag and fiber)

C1= Ash corrected blank bag factor (loss of weight of weight on ignition of blank bag)

3.7. Data Analysis

3.7.1. Statistical Test

A two-way ANOVA was performed to assess the effects of feeding rate, substrate type, feeding regime, and combined substrate levels on BSF larvae growth performance among groups. Post-hoc Analysis Tukey's test was performed at a 95% confidence level to determine the least significant difference in mean separation between treatments. The data was presented as graphs and tables. The normality of data and uniformity of variance among the groups was checked before the decision to use ANOVA for the statistical analysis.

3.7.2. Computations

The BSF larvae's ability to consume and thus reduce organic substrates was determined by calculating waste reduction efficiency, feed conversion into increased body mass efficiency (feed conversion rate, FCR), and bioconversion rate, as described previously (Banks et al., 2014; Diener et al., 2009).

- **Bioconversion rate** (%) = Total larval mass/Total feed added x 100
- **Substrate Reduction** (%) = (Total feed added − Residue feed after treatment)/Total feed added x 100.
- Relative growth rate(%)= (Final larval biomass-Initial larval bimass)/ Initial larval bimass x 100

4. **Results**

4.1. Performance of BSFL fed on different ingredient substrates at different levels of feeding rates

4.1.1. Performance of the BSFL on various parameters

The final larval biomass, relative growth rate, bioconversion rate and substrate reduction rate of the BSFL fed on three different local ingredient bio wastes at different levels of feeding rates are provided in table 3, figures 4 & 5.

Table 3.Summary of the various parameters of BSFL fed on three different local ingredient bio wastes at different levels of feeding rates

		Feeding Rate (g/larva/day)				
Substrate type	Parameter	100	150	200	250	Total
Poultry manure (PM)	Relative growth rate (%)	95.24	94.44	74.28	88.57	88.13
	Final larval biomass (g)	11.67	11	11	11.33	11.25
	Bioconversion rate (%)	12.56	8.36	5.88	4.75	7.89
	Substrate reduction rate (%)	45.63	40.43	42.5	42.45	42.76
	Maturation day	11.67	12.67	12.67	13.33	12.58
Rumen liquor (RL)	Relative growth rate (%)	80.95	55.71	71.42	79.36	71.86
	Final larval biomass (g)	11.33	10.33	9.67	10	10.33
	Bioconversion rate (%)	8.49	5.12	3.99	3.15	5.19
	Substrate reduction rate (%)	40.20	41.81	38.25	42.12	40.59
	Maturation day	15.00	16.33	17.33	17.33	16.50
Brewery waste (BW)	Relative growth rate (%)	103.81	110.71	85.71	97.14	99.34
	Final larval biomass (g)	12.67	12	11.67	12.33	12.17
	Bioconversion rate (%)	8.61	5.62	4.19	3.39	5.45
	Substrate reduction rate (%)	51.22	46.07	45.49	44.22	46.75
	Maturation day	14.67	14.33	15.33	15.67	15.00
Total	Relative growth rate (%)	93.33	86.95	77.14	88.35	
	Final larval biomass (g)	11.89	11.11	10.78	11.22	
	Bioconversion rate (%)	9.89	6.37	4.69	3.76	
	Substrate reduction rate (%)	45.69	42.77	42.09	42.93	
	Maturation day	13.78	14.44	15.11	15.44	

Based on a comparison of the feeding ingredients, a number of features showed differences between them. Figure 4 shows a variation in parameter values between the ingredients, in addition to the information in table 3. Overall, BSFL fed on brewery waste had higher parameter values while those fed on rumen liquor had lower parameter values.

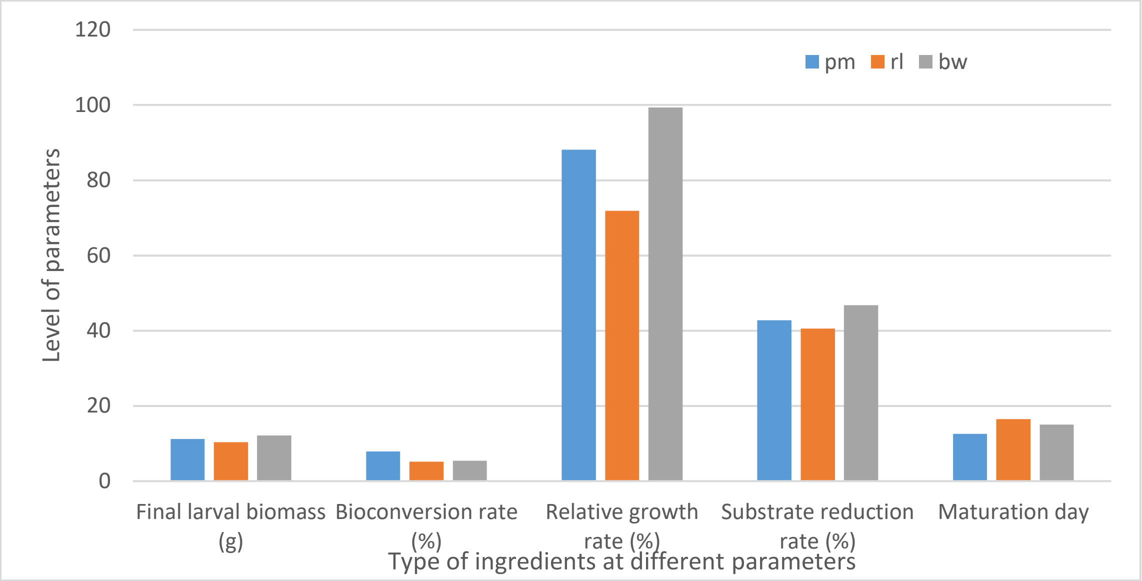

Fig 4. Comparison of the parameters by the biowastes used as feeding substrates for the BSF larvae

The BSFL parameters also revealed differences in terms of feeding rates as shown in Table 3 and Figure 5. The study found that the highest values for the BSFL parameters were recorded at a feeding rate of 100 g/g larvae/day, and the lowest average value were recorded at a feeding rate of 250 g/g larva/day.

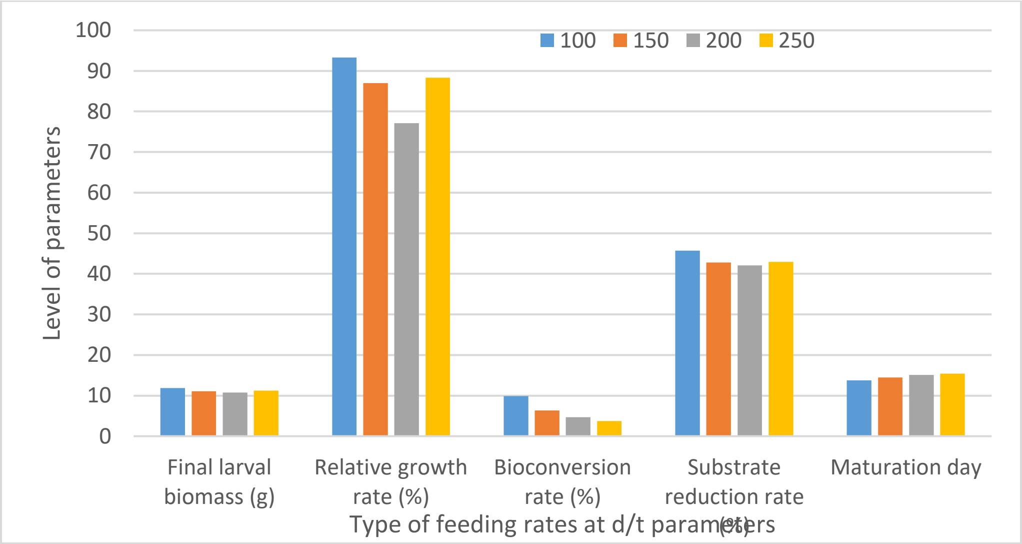

Fig 5. Comparison of the parameters for the BSFL fed on various ingredient substrates at different levels of the feeding rates

4.1.2. Statistical comparisons of the BSFL parameters

The statistical analysis of the BSFL parameters in terms of substrate type and feeding rate are presented below.

Larval Biomass and Relative Growth Rate: Both the relative growth rate and the weight gain were highly influenced by the type of substrate ($p<0.05$) showing the impact of varying sources of nutrients on the development of BSFL. On the other hand, feeding rate did not significantly affect these variables (Table 4, $P >0.05$, Table 3). The Tukey HSD test revealed no statistically significant difference between poultry manure and rumen liquor for both biomass and growth rate (Table 5, $P < 0.05$; Table 3). Conversely, a significant difference exists between Rl and Bw, suggesting that Bw promotes the highest larval biomass and relative growth rate (Table 5, $P <0.05$; Table 3).

Substrate Reduction and Bioconversion rates: The study found that neither substrate type nor feeding rate had an impact on substrate reduction rate (two-way ANOVA, $P >0.05$). However, the type of feeding ingredients and feeding rate had a substantial impact on bioconversion rate ($p<0.05$, Table 3, 4 and 5). Higher values of bioconversion rates were observed in poultry manure ingredients while lower values were observed in rumen liquor. At a feeding rate of 100 grams per gram of larvae per day, the average bioconversion efficiency of the black soldier fly larvae was considerably higher than it was at the other levels.

Maturity Duration: The length of time it takes the BSFL to mature was strongly influenced by both the feeding rate ($P = 0.05$) and substrate type ($P = 0.00$, Table 3 and 4). Tukey HSD test showed that all comparisons between substrate types (Pm, Rl, and Bw) showed significant differences in development time according the. On the other hand, feeding rate had only marginal effect. While there was no significant difference between feeding rates of 150 and 200 or 200 and 250 (Table 5, $P > 0.05$; Table 3), feeding rates from 100 to 200 or 250 significantly differ in development time (Table 5, $P < 0.05$; Table 3)

Table 4. Summary of the statistical analysis (**two-way ANOVA**) for the differences in the mean values of the parameters of BSFL reared on ingredient substrates at different levels of feeding rates.

Parameter/2-way ANOVA	Source of Variation	Df	F-value	P-value
Larval biomass (g)	Substrate type	2	3.82	0.03*
	Feeding rates	3	0.74	0.54
	Interaction effect	6	0.09	0.99
Relative growth rate (%)	Substrate type	2	4.256	0.026*
	Feeding rates	3	0.769	0.523
	Interaction effect	6	0.565	0.754
Bioconversion rate (%)	Substrate type	2	129.63	0.00*
	Feeding rates	3	319.29	0.00*
	Interaction effect	6	6.67	0.00*
Substrate reduction rate (%)	Substrate type	2	2.26	0.13
	Feeding rates	3	0.44	0.73
	Interaction effect	6	0.28	0.94
Maturity durations	Substrate type	2	52.71	0.00*
	Feeding rates	3	5.53	0.052
	Interaction effect	6	0.719	0.68

* Means within a row and column having different values were statistically different at $p<0.05$

Table 5.Summary of the **post-hoc** analysis for the differences in the mean values of the parameters of BSFL reared on ingredient substrates at different levels of feeding rates

Parameters	Groups	P- Value
Larval Biomass (G)	Pm Vs Rl	0.09
	Pm Vs. Rl	0.05
	Rl Vs Bw	0.00*
Relative growth rate Rate (%)	Pm Vs Rl	0.219
	Pm Vs. Bw	0.474
	Rl Vs Bw	0.021*
Bioconversion rate (%)	Pm Vs Rl	0.000*
	Pm Vs. Bw	0.000*
	Rl Vs Bw	0.34
	100 Vs. 200	0.000*
	100 Vs 250	0.000*
	150 Vs. 200	0.000*
	150 Vs. 250	0.000*
	200 Vs. 250	0.001*
Maturation time	Pm Vs Rl	0.000*
	Pm Vs. Bw	0.000*
	Rl Vs. Bw	0.002*
	100 Vs. 150	0.45
	100 Vs 200	0.03*
	100 Vs. 250	0.005*
	150 Vs. 200	0.45
	150 Vs. 250	0.139
	200 vs. 250	0.876

* Means within a row or column having different values were statistically different at $p<0.05$

4.2. Performance of BSFL on combined substrate on various feeding frequencies

4.2.1. Performance of BSFL on various parameters

The final larval biomass, relative growth rate, bioconversion rate and substrate reduction rate of the BSFL fed on three different local ingredient bio wastes at different levels of feeding rates are provided in Table 4, Figures 6 & 7.

Table 6.Summary of the various parameters of BSFL fed on three different Substrates Combinations with different feeding regimes: daily feeding regime (Dlf), midweek feeding regime (Mwf), weekly feeding regime (Wkf) and lump sum feeding rate (Lsf)

Substrate Combination Type	*Parameter*	*Feeding Regimes*				
		Dlf	Mwf	Wkf	Lsf	Total
50:50 (Pm & Rl)	Relative rowth rate (%)	133.33	155.56	144.44	161.11	148.61
	Final larval biomass (G)	7.67	8.33	8.00	8.67	8.17
	Bioconversion rate (%)	12.96	14.20	13.58	14.50	13.81
	Substrate reduction rate (%)	28.24	30.25	27.93	31.33	29.44
	Maturation day	16.67	16.33	17.00	16.67	16.67
50:50 (Pm & Bw)	Relative growth rate (%)	238.89	355.56	266.67	311.11	293.06
	Final larval biomass (G)	11.00	11.67	11.00	12.33	11.50
	Bioconversion rate (%)	18.83	25.31	20.37	26.54	22.76
	Substrate reduction rate (%)	35.34	36.73	35.19	38.89	36.54
	Maturation day	13.33	14.00	14.00	14.00	13.83
75:25 (Pm & Rl)	Relative growth rate (%)	163.89	255.56	205.56	272.22	224.31
	Final larval biomass (G)	8.67	9.00	8.00	9.67	8.83
	Bioconversion rate (%)	14.66	19.75	16.97	20.68	18.02
	Substrate reduction Rate (%)	29.32	33.64	29.63	33.33	31.48
	Maturation day	14.67	15.33	15.67	15.33	15.25
Total	Relative growth rate (%)	178.70	255.56	205.56	248.15	
	Final larval biomass (G)	9.11	9.67	9.00	10.22	
	Bioconversion rate (%)	15.48	19.75	16.97	20.57	
	Substrate reduction rate (%)	30.97	33.54	30.92	34.52	
	Maturation day	14.89	15.22	15.56	15.33	

Among the different substrate combinations tested, the best performance of black soldier fly larvae (BSFL) was observed when poultry manure and brewery waste were mixed in equal proportions. This was followed by a mixture of poultry manure and rumen liquor in a 3:1 ratio, with the combination of poultry manure and rumen liquor in equal proportions resulting in the lowest BSFL performance.

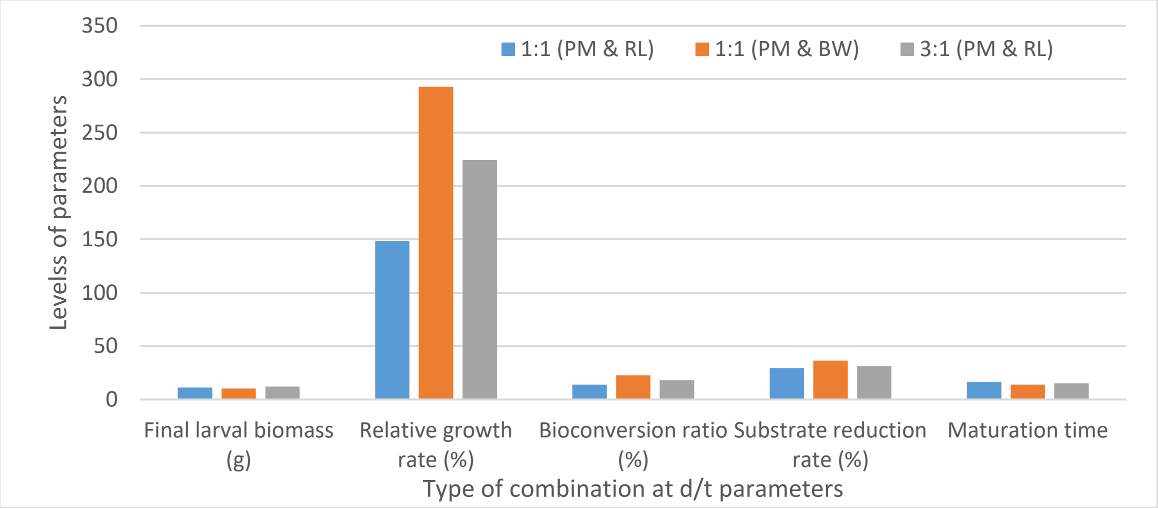

Fig 6. Comparison of the parameters for the BSFL fed on various ingredient substrates at different levels of the feeding rates substrates.

Figure 7 illustrates the large difference in the influence of feeding frequency on all evaluated parameters (larval biomass, relative growth rate, bioconversion rate, substrate reduction rate, and maturity time).The Black Soldier Fly Larvae (BSFL) performed the best on all parameters when fed in a lump sum, followed by the mid-weekly feeding regime.

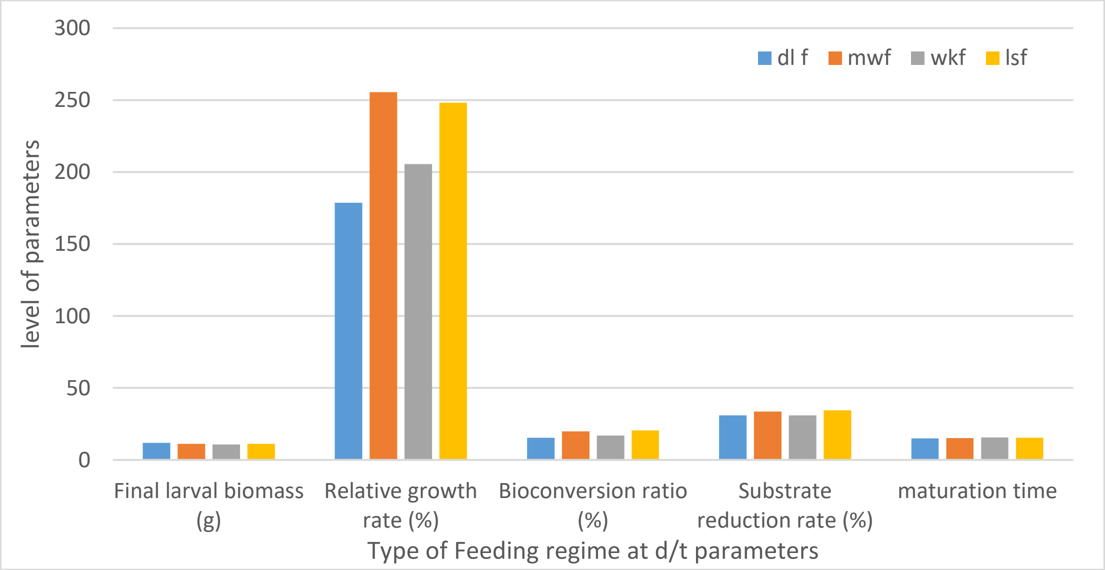

Fig 7. Comparison of the parameters for the BSFL fed on various combinations of substrates at different feeding frequencies

4.2.2. Statistical comparisons of the BSFL parameters

Larval Biomass and Growth Rate: The specific type of mixed substrate had a significant impact on larval biomass (weight gain) and relative growth rate (p-value < 0.05, Tables 6 and 7) Table 8 shows that there are no significant differences in larval biomass between Pm: Rl (75:25) and Pm: Rl (50:50) substrate combination types.Feeding frequency did not significantly affect relative growth rate (p-value > 0.05), but had a significant influence on larval biomass (p-value < 0.05, see Tables 6 and 7).

Bioconversion and Substrate Reduction: Substrate combination types had a significant impact on both bioconversion and substrate reduction, with Pm: Rl (50:50) vs. Pm: Bw (50:50) and Pm:Bw (50:50) vs. PM:RL (75:25) at p-value < 0.05. Feeding frequency also showed variations in substrate reduction rate (p-value < 0.05), but no significant variation in bioconversion (p-value > 0.05, see Tables 6 and 7). Substrate combination types had a considerable effect on both bioconversion and substrate reduction. However, there was no significant variation in substrate reduction for some substrate groups (p-value > 0.05, Table 8).

Duration of Maturity: The larval maturity time was strongly influenced by the kind of substrate (p-value < 0.05. however, feeding frequency and its interaction with the substrate type were not statistically significant (p-value > 0.05, Tables 6 and 7).

Table 7. Summary of the statistical analysis (two-way ANOVA) for the differences in the mean values of the parameters of BSFL reared on combinations of substrates at different levels of feeding frequencies.

Parameter/2-way ANOVA	*Source of Variation*	*Df*	*F-value*	*P-value*
Larval biomass (g)	Substrate combination	2	53.76	0.00*
	Feeding frequency	3	4.1	0.01*
	Interaction effect	6	0.26	0.91
Relative growth rate (%)	Substrate combination	2	14.02	0.00*
	Feeding frequency	3	2.65	0.07
	Interaction effect	6	0.42	0.86
Bioconversion rate (%)	Substrate combination	2	14.45	0.00*
	Feeding frequency	3	3.05	0.056
	Interaction effect	6	0.45	0.84
Substrate reduction rate (%)	Substrate combination	2	18.05	0.00*
	Feeding frequency	3	3.38	0.03*
	Interaction effect	6	0.16	0.98
Maturity time	Substrate combination	2	61.92	0.00*
	Feeding frequency	3	1.78	0.17
	Interaction effect	6	0.5	0.81

* Means within a row or column having different values were statistically different at p<0.05

Table 8.Summary of the **post-hoc** analysis for the differences in the mean values of the parameters of BSF larvae reared on ingredient substrates at different levels of feeding frequency

Parameters	Groups	P- Value
Larval biomass (g)	Pm:Rl(50:50) Vs Pm:Bw (50:50)	0.000*
	Pm:Bw (50:50) Vs Pm:Rl (75:25)	0.000*
	Pm:Rl (75:25) Vs Pm:Rl(50:50)	0.062
	Dlf Vs Mwf	0.170
	Dlf Vs Wkf	0.780
	Dlf Vs Lsf	0.009*
	Mwf Vs Wkf	0.103
	Mwf Vs Lsf	0.170
	Wkf Vs Lsf	0.005*
Substrate reduction rate (%)	Pm:Rl(50:50) Vs Pm:Bw (50:50)	0.000*
	Pm:Bw (50:50) Vs Pm:Rl (75:25)	0.001*
	Pm:Rl (75:25) Vs Pm:Rl(50:50)	0.23
Bioconversion rate (%)	Pm:Rl(50:50) Vs Pm:Bw (50:50)	0.000*
	Pm:Bw (50:50) Vs Pm:Rl (75:25)	0.023*
	Pm:Rl (75:25) Vs Pm:Rl(50:50)	0.047*
Relative growth rate (%)	Pm:Rl(50:50) Vs Pm:Bw (50:50)	0.000*
	Pm:Bw (50:50) Vs Pm:Rl (75:25)	0.048*
	Pm:Rl (75:25) Vs Pm:Rl(50:50)	0.027*
Maturity duration	Pm:Rl(50:50) Vs Pm:Bw (50:50)	0.000*
	Pm:Bw (50:50) Vs Pm:Rl (75:25)	0.000*
	Pm:Rl (75:25) Vs Pm:Rl(50:50)	0.000*

* Means within a row or a column having different values were statistically different at $p<0.05$

4.3. Proximate compositions

The substrates showed higher levels of ash and fiber compared to the BSFL tissue. Conversely, the BSFL tissue contained higher amounts of crude protein and crude fat than the substrates. When grown on brewery waste, BSFL had higher percentages of crude protein and crude fat on ingredient substrates. On combined substrates, BSFL grown on a 50:50 mix of Pm: Bw had higher percentages of crude protein and crude fat (Table 9).

Table 9.Proximate composition of substrates used for the BSFL experiments and larvae reared on different substrates. CP = crude protein, CF = crude fat

	Substrate and larvae	%CP	%CF	%Ash	%Fiber
Substrate	Rumen liquor	18.9	3	33.3	16.6
	Poultry manure	23.5	4.3	34.2	25.6
	Brewery waste	26.3	6.8	32.6	16.59
Larvae per substrate	Larvae, Pm	39.7	27	9.008	10.4
	Larvae, Bw	44.5	31.2	9.8	11.2
	Larvae, Rl	41.5	28.1	10.009	9.9
	Larvae, Pm:Rl (50:50)	42.5	28.5	16.7	8.1
	Larvae, Pm:Bw (50:50)	46.5	32.41	12.4	6.9
	Larvae, Pm:Rl (75:25)	44.3	29.3	12.8	7.9

Brewery waste has the largest CP concentration, whereas poultry dung contains the highest percentage of ash. The larvae reared on a brewery waste diet have the highest CP and CF content. The practice of combining substrates has the potential to impact the ultimate larval makeup. The Larvae reared on Pm:Bw (50:50) has a greater CP and CF content than larvae among the others.

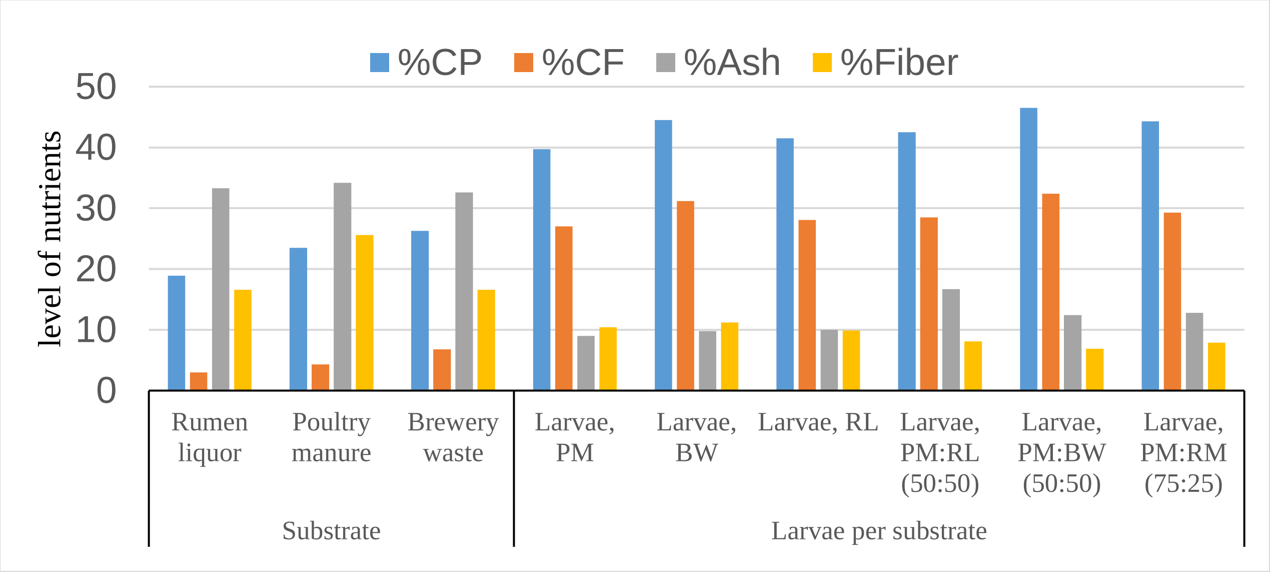

Fig 8. Substrate composition and larvae composition

5. Discussion

5.1. Performance of BSFL fed on different ingredient substrates at different levels of feeding rates

5.1.1. Larval biomass (g)

This study investigated the impact of ingredient feed types and feeding rates on larval biomass and relative growth rates of black soldier fly larvae (BSFL). The results revealed that the total larval biomass was significantly influenced by the type of ingredients used, rather than by the feeding rate. Among the ingredients tested, brewery waste (Bw) yielded the highest larval production, followed by poultry manure (Pm), while rumen liquor (Rl) resulted in the lowest larval biomass. Specifically, Bw had an average larval yield of 12.2 ± 1.03 g, Pm had 11.3 + 1.7 g, and Rl had 10.3 + 1.78 g ($p = 0.03$, Table 3). Regarding feeding rates, there was no significant variance in mean larval biomass. The largest larval yield occurred at a feeding rate of 100 g feed/g larvae/day, followed by 200 g feed/g larvae/day, and the lowest at 150 g feed/g larvae/day. The average larval yields were 11.2 mg, 11.11 mg, and 10.78 mg ($p = 0.54$, Tables 3 and 4). Brewery waste at feeding rate of 100 mg/larva/day seems optimal for maximizing larval weight, as we compared to Nyakeri et al. (2017) the maximum average weight of 0.101 g and the minimum average weight 0.055 g.

5.1.2. Relative Growth Rate

Relative growth rate was also influenced by ingredient types rather than feeding rates. This shows a significant impact of varying sources of feed nutrients on the development of BSFL. Based on this, the biggest relative growth rate was observed in brewery waste (99.34%), followed by poultry manure (88.13%), and the lowest value was observed in rumen liquor (71.86%) (Table 3, $p = 0.026$). On the other hand, feeding rate did not significantly affect these variables, but there are slight differences between them. The highest relative growth rate was observed at feeding rates of 100 g feed /g larvae/day (93.3%), while the lowest value was observed at the 200 g feed/g larvae/day feeding rate's (77.14%), according to Table 4 ($P > 0.05$) analysis. The best relative growth rate and larval biomass may be observed due to the nutritional impacts. Brewer's waste (BW) is also a mixture of spent grains and yeast cells used for grain fermentation and has a proximate composition of 12.2% crude fiber and 26.8% crude protein (Munguti et al., 2006). Because of their greater nutritional content and varied nature, BW was found to have higher mean larval biomass and yield.

5.1.3. Bioconversion rate

The efficiency of black soldier fly larvae (BSFL) in converting organic matter into biomass is influenced by several factors, including the type of feedstock, feeding rate, and potential interactions between different substrates (Barragan et al., 2017). Previous studies have reported varying bioconversion rates, expressed as dry larval biomass produced per unit of waste. For instance, Gold et al. (2018) and Liu et al. (2018) found that bioconversion rates ranged from 1.9% to 6% for animal manure substrates, 5% for brewery byproducts, and 4-5% for fruit and vegetable waste (Lalander et al., 2019; Somroo et al., 2019). Notably, food waste substrates exhibit significantly higher bioconversion rates, reaching up to 21% (Nyakeri et al., 2017; Lalander et al., 2019).

The findings of this current investigation demonstrate promising results in comparison to existing literature. Bioconversion rates were consistently higher for poultry manure compared to rumen liquor, with a maximum value of 7.89% and a minimum of 5.19%. Feeding rate also significantly impacted on bioconversion efficiency. The highest rate was observed at 100 g feed/g larvae/day, followed by 150 g feed/g larvae/day. The lowest bioconversion rate occurred at the highest feeding rate of 250 g feed/g larvae/day (p=0.00, Tables 4 and 5).

5.1.4. Substrate matter reduction efficiency

This is meant to give an indication of whether the substrate is sufficiently fed on and therefore reduced by the larvae to achieve suitable biomass within an acceptable duration. A low substrate reduction value indicates wastage of the substrate, either because it is not consumed or because it is in excess. A very high reduction value, on the other hand, indicates possible starvation of the larvae at the feeding ration being used (Mutafela, 2015). However, there was no observable significance variance between waste reduction rates data in terms of feeding ingredients and feeding rates in Table 4, but the maximum substrate reduction rates were observed on Bw with 46.75%, followed by Pm with 42.76, and the minimum value was observed on RL with 40.59%. Also, the optimum values of substrate reduction rate were observed at feeding rates of 100 g/day (45.69%) and the minimum values were observed at feeding rates of 200 g/day.

However, there was no visible significant variance in waste reduction rates data in terms of feeding ingredients and feeding rates in Table 4, yet there was not a significant distinction between waste reduction rates data in terms of feeding ingredients and feeding rates in Table 4, but the largest substrate reduction rates were found on Bw (46.75%), followed by Pm (42.76%), and the least value was observed on Rl (40.59%).

Furthermore, the maximum substrate reduction rate was recorded at feeding rates of 100 g/day (45.69%), while the least was observed at feeding rates of 200 g/day (42.09%). A higher feeding rate can result in faster growth and more BSF biomass output, but it can also reduce the rate of biomass conversion and waste reduction (Barragan et al., 2017; Diener et al., 2009). However, a combination of high larval density and a high feeding rate has a negative impact on waste reduction and the larvae's relative development rate (Parra Paz et al., 2015).

5.1.5. Maturation time

This study revealed a significant influence ($p < 0.05$) of both feed type and feeding rate on the maturation time of black soldier fly larvae (BSFL) (Tables 3 and 4). This variation is likely attributable to the distinct nutritional profiles of the feed ingredients and the feed quantity relative to the larval population density. Poultry manure promoted the fastest larval development, with a maturation time of only 13 days. Conversely, rumen liquor resulted in the slowest maturation, requiring 17 days for larvae to reach maturity. Interestingly, feeding rate exhibited an inverse relationship with maturation time. Larvae fed at a rate of 100 g feed/g larvae/day matured in 14 days, while those fed at a higher rate of 250 g feed/g larvae/day took 16 days.

It is interesting to note that several research have observed varying maturation times for BSFL cultivated on poultry manure. Rehman et al. (2017) found a significantly longer maturation time ranging from 18.57 to 21.36 days, while Zhang et al. (2023) reported an extraordinarily rapid development period of about 10 days. The current study's finding of 13 days for manure-based diets falls within this established range. This intermediate value suggests a potential "optimal growth strategy" where the chosen manure and feeding regime may offer a balance between faster development and other critical performance metrics, such as larval survival and bioconversion efficiency.

5.2. Growth performance of BSFL on substrate combination level and feeding frequency

5.2.1. Larval biomass (g)

This study investigates the influence of substrate composition and feeding regimen on larval biomass and growth rates. A 50:50 mixture of poultry manure (Pm) and brewer's waste (Bw) yielded the highest larval biomass (11.05 g), suggesting a synergistic effect on larval development. Conversely, the combination of Pm and rumen liquor (Rl) resulted in the lowest biomass (8.2 grams), indicating a potential nutritional deficiency in Rl for optimal larval growth (tables 7 and 8, $p<0.05$).

This result shows a positive result as we compare it with Nyakeri et al. (2017), who state that FR gave significantly higher average larval weights of 0.101±0.002 g, while the lowest average larval weight was 0.055±0.002 g. Also, the larval biomass for Bw and FS was 0.078 and 0.070 g, respectively, and Adebayo et al. (2021) state that among the various substrates, CF had the greatest larval weight (0.30 g), length (2.18 cm), and quickest development period (21 days). Feeding frequency also emerged as a significant factor. Larvae offered a single, lump sum diet that exhibited the greatest average biomass (10.22 grams), followed by midweek feeding (9.78 grams) and weekly feeding (9.0 grams) ($p = 0.00$, Tables 6 and 7). This result was really correlated with Mutafela's (2015) continuous feeding mode, which also recorded lower larval biomass compared to the batch mode.

5.2.2. Relative Growth Rate

The studied data from two ANOVAs showed that mixed substrates had a greater effect on the relative growth rate of black soldier fly larvae than feeding frequency ($p = 0.00$, table 7). The results of this investigation showed that the substrate mixture of Pm and Rm (50:50) had the lowest relative growth rate, while the substrate mixture of Pm and Bw (50:50) had the highest value. In terms of their order from maximum to minimum points, the average relative growth rates were 293.06% and 224.31%, respectively, while the lowest value was 148.61%. Table 6 shows that the variance of this value was statistically significant. However, there is no statistically significant difference in the impact of the feeding regime on the relative growth rates. The lump sum feeding frequency (248.15%) exhibited the best value, while the daily feeding frequency (178.7%) showed the lowest value ($p > 0.05$, Table 6).

5.2.3. Bioconversion ratio and substrate reduction

The type of mixed substrate significantly influenced both bioconversion efficiency and substrate reduction rates in black soldier fly (BSFL) larvae ($p < 0.00$, Table 6). Interestingly, feeding frequency only impacted substrate reduction, not bioconversion. A 1:1 mixture of poultry manure (Pm) and brewer's waste (Bw) achieved the highest bioconversion rate (22.78%), followed by a notable decrease with the 1:1 Pm and rumen liquor (Rl) mixture (13.81%) (Table 6). Feeding frequency exhibited no statistically significant effects; however, the single, large feeding approach attained the highest bioconversion rate (20.57%), while daily feeding resulted in the lowest (15.48%) (Table 6).

Substrate reduction rates mirrored the trends observed in bioconversion. The 1:1 Pm and Bw mixture again demonstrated the greatest reduction (36.54%), highlighting its superior efficacy in substrate utilization.

The 3:1 Pm and Rl mixture displayed a slightly higher reduction rate (31.48%) compared to the 1:1 Pm and Rl mixture (29.44%), suggesting potential limitations in Rl suitability for larval development. Regarding feeding regimes, the lump sum feeding method yielded the highest reduction rate (34.52%), while the weekly feeding regime resulted in the lowest (30.92%) ($p < 0.05$, Tables 7 and 8). This variation was occurred not only depend on substrate type, rather it was also depend on larval density and feed quantities (Parra Paz et al., 2015). These findings align with previous research by Diener et al. (2009, 2011) and Parra Paz et al. (2015), emphasizing that larval density, feeding rate, and frequency are all critical factors influencing substrate conversion efficiency. Encouragingly, the bioconversion rates observed in this study surpassed those reported in existing literature for similar substrates (Rehman et al., 2017; Lie et al., 2022). The observed range (6.3- 22.78% for organic waste and 13.04 - 18.54% for mixed substrates) suggests a potentially enhanced effectiveness of the employed BSFL population in both consuming and reducing substrate biomass.

5.2.4. Duration of Maturity

The larval maturity time was strongly influenced by the kind of mixed substrate. The results of this investigation showed that the substrate mixture of Pm and Rl Bw (50:50) had the smallest value (14 days) ($p = 0.00$, Tables 6 and 7). While feeding frequency and their interaction were not statistically significant (p-value > 0.05, Tables 6 and 7).The performance of BSFL can be greatly impacted by different combinations, which are likely to have differing nutrient profiles and physical features. Among the different substrate combinations tested (Fig. 6), the best performance of black soldier fly larvae (BSFL) was observed when poultry manure and brewery waste were mixed in equal proportions. This was followed by a mixture of poultry manure and rumen liquor in a 3:1 ratio, with the combination of poultry manure and rumen liquor in equal proportions resulting in the lowest BSFL performance.

5.3. Proximate compositions analysis

The quality and quantity of food that the larvae consume determine their body composition (Gobbi, 2012; Newton et al., 2005; Nguyen et al., 2015). The body composition of BSF larvae varies among substrates not only in protein content (37 to 63% dry matter; DM), but also in fat content, which varies the most (7 to 39% DM) (St Hilaire et al., 2007; Zheng et al., 2012).

In this study, the larvae showed a variety of crude protein, fat content, ash, and fiber values ranging from 27% to 32.41%, 9.01% to 16.7%, 6.9% to 12.8%, and 7.9% to 11.2%, respectively, in the experiment that used different substrates.The values for crude protein in the range of 27% to 32.41% (Tables 9 and Fig. 8) are somehow better than those reported elsewhere, such as 15–25% for larva fed on poultry manure (Gutiérrez et al., 2004) and 25% for swine manure (Newton et al., 2005). However, they are somehow lower than 35% for cattle manure (Newton et al., 1977) and 42-49% for oil-rich food waste (Barry, 2004). The crude fat content seems to be dependent on the diet used for larval production, while the CP content is fairly constant and may be related to genetics (Table 9 and Fig. 8). St Hilaire et al. (2007) also reported that the fat quality was dependent on the diet type, where elevated levels of omega-3 fatty acids, which are essential for fish and human health, were observed in larvae fed on fish scraps.

The larvae raised on different substrates had a higher percentage of CP than the substrates themselves. This suggests that Black Soldier Fly Larvae (BSFL) were efficient in converting substrates into body proteins. The highest CP was discovered in larvae raised on a combination of poultry manure and brewery waste (46.5%).Similar to CP, larvae had higher CF than the substrates. Larvae fed on brewery trash had the highest CF content (31.2%), indicating that this substrate may be high in lipids or that the larvae favor lipid accumulation when given brewery waste. The ash percentage was generally lower in larvae than in substrates, with the exception of larvae raised on a 50:50 mix of poultry manure and rumen fluid, which had a much higher ash content (16.7%). This could be because the mineral content of the substrates was being absorbed by the larvae's body tissue. The fiber content in larvae was consistently lower than in substrates. This could indicate that BSFL are unable to digest fiber well or that they prefer to take other nutrients over fiber.

6. Conclusion and recommendations

6.1. Conclusion

Overall, the investigation of black soldier fly larvae (BSFL) performance on three locally available component substrates and four different feeding rates found that brewery waste and poultry dung gave positive and comparable results. Feeding rate had little effect on BSFL properties. However, feeding at 100 grams per gram of larvae each day appears to be more advantageous than the other feeding rates. Among the various substrate combinations studied, the optimal performance of black soldier fly larvae (BSFL) was identified when poultry manure and brewery waste were mixed in equal proportions during lump sum feeding regimes, followed by a 3:1 mixture of chicken manure and rumen liquor, with the lowest BSFL performance achieved by combining poultry manure and rumen liquor in the same ratio. The daily and mid-weekly feeding regimens showed roughly comparable impacts on all BSFL parameters, with the lowest overall effect. The crude protein and fat content of larvae from various substrates correlated strongly with the nutritional level of the feedstock used

6.2. Recommendations

While the 50:50 Pm & Bw mix shows promise, exploring other combinations of locally available biowastes to potentially optimize production costs and efficiency is recommended.

Investigate the optimal nutrient ratios within substrate mixtures for further biomass and growth rate improvements

BSF production for aquaculture feed offers a sustainable and economical solution for farmers and the agricultural sector. So we recommend that incorporating BSF into your operations, you can contribute to a more circular food system and improve the health of your fish and for other animal feed such as chicken and pigs.

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8. Appendix PART 1:

DIFFERENT IMAGES

Figure I.Attractant preparation and processing

Life Cycle of the Black Soldier Fly and Reproductions

The BSF has five life stages: egg, larvae, prepupae, pupae, and adult. BSF has an estimated life cycle of 40 days, but this varies depending on the environmental conditions and nutrition provided (Alvarez, 2012)

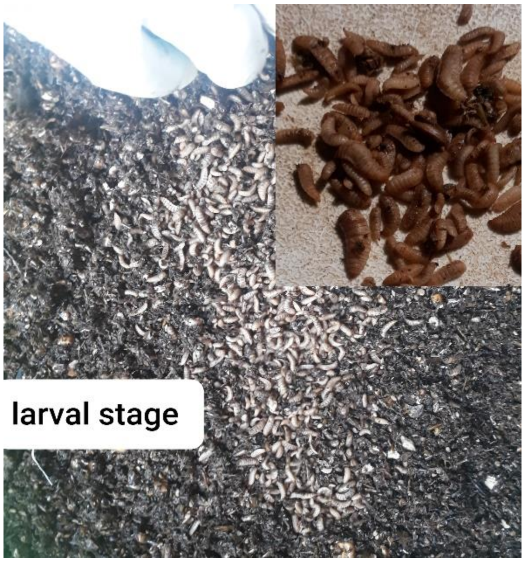

Figure II. Life cycle of a black soldier flay

Figure III. Eggies in adult cage (left side) and incubator for hatching eggs (right side)

PART. 2: TABLES OF ANALYSIS

Table I. ANOVA Tests of Between-ingredient type and feeding rates

Dependent Variable: larval biomass

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Ingredient type	20.167	2	10.083	3.821	.036
Feeding rates	5.861	3	1.954	.740	.538
Ingredient type * feeding rates	1.389	6	.231	.088	.997
Error	63.333	24	2.639		
Total	4647.000	36			
Corrected Total	90.750	35			

a. R Squared = .302 (Adjusted R Squared = -.018)

Table II. Post hockey analysis between ingredients

Dependent Variable: larval Biomass

Tukey HSD

(I) ingredient type	(J) ingredient type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
PM	RL	.92	.663	.366	-.74	2.57
	BW	-.92	.663	.366	-2.57	.74
RL	PM	-.92	.663	.366	-2.57	.74
	BW	-1.83*	.663	.028	-3.49	-.18
BW	PM	.92	.663	.366	-.74	2.57
	RL	1.83*	.663	.028	.18	3.49

Based on observed means.The error term is Mean Square(Error) = 2.639.

*. The mean difference is significant at the .05 level.

Table III. Correlations between ingredient type and feeding rates

		ingredient type	feeding rates
ingredient type	Pearson Correlation	1	.000
	Sig. (2-tailed)		1.000
	N	36	36
feeding rates	Pearson Correlation	.000	1
	Sig. (2-tailed)	1.000	
	N	36	36

Table IV. ANOVA test between combination type vs feeding frequency

Dependent Variable larvall biomass

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	84.333[a]	11	7.667	11.040	.000
Intercept	3249.000	1	3249.000	4678.560	.000
Combination type	74.667	2	37.333	53.760	.000
F frequency	8.556	3	2.852	4.107	.017
combtype * ffrequency	1.111	6	.185	.267	.947
Error	16.667	24	.694		
Total	3350.000	36			
Corrected Total	101.000	35			

a. R Squared = .835 (Adjusted R Squared = .759)

Table V. Post hoc test between substrate combinations

Dependent Variable larval biomass

(I) comb type	(J) comb type	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1:1 pm &rl	1:1 pm & bw	-3.333*	.340	.000	-4.035	-2.631
	3:1 pm &rl	-.667	.340	.062	-1.369	.035
1:1 pm & bw	1:1 pm &rl	3.333*	.340	.000	2.631	4.035
	3:1 pm &rl	2.667*	.340	.000	1.965	3.369
3:1 pm &rl	1:1 pm &rl	.667	.340	.062	-.035	1.369
	1:1 pm & bw	-2.667*	.340	.000	-3.369	-1.965

Based on estimated marginal means

*. The mean difference is significant at the 0.05 level. b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table VI. Post hockey analysis between feeding regimes

Dependent Variable larvall biomass

LSD

(I) f frequency	(J) f frequency	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
DLF	MWF	-.56	.393	.170	-1.37	.26
	WKF	.11	.393	.780	-.70	.92
	LSF	-1.11*	.393	.009	-1.92	-.30
MWF	DLF	.56	.393	.170	-.26	1.37
	WKF	.67	.393	.103	-.14	1.48
	LSF	-.56	.393	.170	-1.37	.26
WKF	DLF	-.11	.393	.780	-.92	.70
	MWF	-.67	.393	.103	-1.48	.14
	LSF	-1.22*	.393	.005	-2.03	-.41
LSF	DLF	1.11*	.393	.009	.30	1.92
	MWF	.56	.393	.170	-.26	1.37
	WKF	1.22*	.393	.005	.41	2.03

Based on observed means.The error term is Mean Square(Error) = .694.

*. The mean difference is significant at the 0.05 level.

Table VII. Correlations between substrate combination and feeding regime

		combination type	feedings frequency
combination type	Pearson Correlation	1	.000
	Sig. (2-tailed)		1.000
	N	36	36
f frequency	Pearson Correlation	.000	1
	Sig. (2-tailed)	1.000	
	N	36	36