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NUTRIENT PROFILE, PROTEASE AND CELLULASE ACTIVITIES OF PROTEIN EXTRACTED FROM BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE REARED ON VARIOUS SUBSTRATES

Titin WIDIYASTUTI✉, Sri RAHAYU, Wardhana SURYAPRATAMA, Fransiska Maria SUHARTATI

Faculty of Animal Science, University of Jenderal Soedirman, Jl. Dr. Soeparno Karangwangkal Purwokerto, Indonesia

✉Email: titin.widiyastuti@unsoed.ac.id

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ABSTRACT: The Black Soldier Fly (BSF; *Hermetia illucens*) larvae are recognized for their ability to convert diverse organic materials into protein-rich biomass, depending on the substrate they consume. The composition of these substrates can significantly impact the nutrient profile and enzyme activities of the resulting maggot protein extract (MPE). Therefore, this exploratory research aimed to assess the nutrient content, protease, and cellulase activity of MPE obtained from BSF maggots reared on different substrates, with a specific focus on substrates A (comprising restaurant waste and rejected milk), B (layer manure), and C (kitchen waste). The results showed that maggot meal from layer manure had the highest protein content (45.36%) and the lowest fat content (18.44%). Amino acids in maggot meal contained high levels of glutamic acid, aspartic acid, alanine, valine, leucine, and isoleucine. Lauric acids were found in maggot meal from kitchen waste (33.79%), layer manure (32.18%), and restaurant waste and rejected milk (22.94%). Maggot meal from layer manure had the highest oleic acid content (15.13%). The protein concentration of MPE from various substrates ranged from 0.56 to 0.601 mg/ml (at 60% w/v ammonium sulfate saturation) and 0.555 to 0.609 mg/ml (at 70% ammonium sulfate saturation). The protease activity of MPE from layer manure substrates exhibited optimum activity and stability in neutral to alkaline pH, with activity levels of 0.748 U/mg at pH 7.0 and pH 11.0 (at 60% w/v ammonium sulfate saturation) and 0.774 units/mg at 70% w/v ammonium sulfate saturation. The highest cellulase activity was found in MPE from kitchen waste, which remained stable at pH 5.0-11.0. In general, maggots from different substrate sources exhibited distinct nutrient profiles and enzyme activities. Protein extract from maggots grown in layer manure showed the most suitable nutrient profile for use as an alternative source of protein feed and protease enzymes.

Keywords: Amino acid, Chemical profile, Enzymes, Fatty acid, Maggot.

INTRODUCTION

Maggots are insect larvae that rapidly decompose waste substances, such as kitchen waste (Green and Popa, 2012), straw (Zheng et al., 2012; Liu et al., 2021), as well as manure (Lalander et al., 2013; Banks et al., 2014). The nutrient composition of the body and the survival rate of larvae are strongly influenced by the quality and quantity of larval development media (Gobbi et al., 2013; Makkar et al., 2014). The nutrient composition of the larval body and the survival rate of the larvae are profoundly shaped by the characteristics of the larval development media, both in terms of its quality and quantity. This influence has been noted in previous studies (Gobbi et al., 2013; Makkar et al., 2014) which emphasize that the nutritional makeup and overall well-being of the larvae are significantly dependent on the specific attributes and abundance of the media in which their development takes place.

Katayane et al. (2014) reported that the protein content and dry weight of larvae reared on palm kernel cake or hereinafter reffered to as palm kernel cake (PKC) media were higher than those reared on faecal dung media. This is presumably because the quality of poultry waste protein is lower due to high levels of non-protein nitrogen (NPN: Nitrogen compounds that are not part of proteins, often found in faecal matter) in faeces compared to the PKC (Arief et al., 2012). Several studies have reported that substrates with low nutritional quality will produce fewer Black Soldier Fly (BSF or Black Soldier Fly: A type of fly whose larvae are known for decomposing organic waste) larvae because the growth media contains fewer or limited nutrients (Tschirner and Simon, 2015; Suciati and Faruq, 2017; Maulana et al., 2021). The high production of organic waste in Indonesia is a potential source of substrates for rearing maggots. Maggot meal, characterized by its high protein and fat content, serves as a viable replacement for fish meal in poultry diets, offering an alternative source of nutrition for poultry.

The high protein and fat content of maggots, as highlighted by Rambet et al. (2015), underscores the advantages of using them as a feed source. It is reported that maggot meal (*Hermetia illucens*) contains nutrients, including 35-57% crude protein and 15-49% crude fat (Dordević et al., 2008; Bosch et al., 2014; Abduh et al., 2022), as well as 2.63% calcium and 0.28% phosphorus (Dengah et al., 2015). The quantities of amino acids and the types of fatty acids present in Black Soldier Fly (BSF) larvae are significantly influenced by the specific makeup of the fly's food (Shumo et al., 2019;

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Spranghers et al., 2017). At the same time, the composition of the larvae's diet has an impact on the diverse quantities of amino acids and fatty acids (Tschirner and Simon, 2015; Spranghers et al., 2017) and but not by the different substrate media where the larvae are grown.

The larvae are esteemed for their remarkable nutritional attributes, with a particular emphasis on the substantial quantities and exceptional quality of both their protein and fat content. This unique combination of protein and fat characteristics positions them as highly valuable constituents when incorporated into animal feed formulations, offering a distinct advantage in enhancing the nutritional quality of such feeds and contributing to the overall health and well-being of the animals that consume them (Wang et al., 2017; Wang and Shelomi, 2017; Gold et al., 2018). Maggot flour derived from BSF larvae is considered a viable and appropriate component for animal feed due to its rich content of crucial amino acids, essential fats, and calcium, all of which are vital for promoting and sustaining the growth of livestock. However, the high chitin content in maggot flour has rendered it unpopular as poultry feed. Chitin, a polymer of N-acetyl-D-glucosamine and a small amount of D-glucosamine, is challenging for poultry to digest (Rinaudo, 2006; Hahn et al., 2018; Van Huis, 2020). Additionally, chitin constitutes 8-24% of maggot biomass (Dossey et al., 2016; Soetemans et al., 2020). Numerous researchers have observed that incorporating insect-derived elements into animal feed typically leads to a decrease in the performance of poultry. Elevating the proportion of maggot meal within the animal feed, particularly when reaching levels of 10% and beyond, has been observed to lead to a decrease in the average daily gain (ADG) in avian species, as documented by (Moula and Detilleux, 2019). This dietary adjustment has also been associated with a reduction in egg weight and feed consumption, while simultaneously resulting in an increase in feed conversion rates, as demonstrated in Widjastuti et al. (2014). Hence, the separation of chitin from maggot biomass via protein extraction is a required step.

The BSF larvae's capability to transform organic waste can be attributed to their innate aptitude for generating both protease and cellulase enzymes within their gastrointestinal system. These enzymes enable them to effectively break down proteins and cellulose present in the organic matter used as their rearing substrate. A multitude of hydrolytic enzymes are synthesized by diverse bacterial populations inhabiting the gastrointestinal tract of BSF larvae (Dong et al., 2009; Yu et al., 2011). In the study conducted by Kim et al. (2011), it was revealed that the digestive extracts obtained from BSF larvae's gastrointestinal system exhibited elevated levels of amylase, lipase, and protease enzymatic activities. Maggot protein extract contains proteases and cellulases, which, when added to feed, are expected to enhance feed digestion in the poultry digestive tract and improve feed efficiency. However, there has been limited information regarding protease and cellulase activity in protein extracts. Given this, this study aims to explore protein extracted from BSF larvae, along with its essential nutritional profiles, protease, and cellulase activity. The extracted protein is further investigated for its specific use as poultry feed. The application of maggot protein extract as a feed supplement serves a dual purpose, acting both as a protein supplement and as hydrolytic enzymes that optimize the digestion and absorption processes in the poultry digestive tract.

This study ventures into this uncharted domain, aiming to uncover the intricate interplay between the specific protein compositions inherent in black soldier fly (BSF) larvae and their suitability as a functional feed. Diverging from conventional research approaches that predominantly focused on broader facets such as nutrient content, enzymatic activities, and the overall nutritional profile of the larvae, our investigation hones in on the specific exploration of protein profiles. By shedding light on these profiles and their potential implications in the domain of functional feed, this research aspires to establish a foundational comprehension that may herald innovative strategies in animal nutrition and feed development. This shift in emphasis towards the nuanced intricacies of protein profiles represents a valuable opportunity to delve deeper into uncharted realms and expand our understanding of the black soldier fly larvae's potential role as a substantive protein source in animal diets.

MATERIALS AND METHODS

This exploratory research was conducted using BSF maggots reared in various substrates, categorized into three groups: A (restaurant waste and rejected milk), B (laying hens' manure), and C (kitchen waste). The subjects under observation were BSF maggots within the age range of 10 to 15 days, a phase corresponding to their pre-pupae development, with a physical length ranging from 10 to 15 millimeters. The research implementation has followed the procedures outlined in the research guidelines provided by the Directorate General of Higher Education, Ministry of Education and Culture of the Republic of Indonesia in 2022, as stated in the Decision Letter (SK) number 2127/UN23/PT.01.02/2022. The study followed the guidelines for the ethical treatment of animals as set forth, ensuring that no undue harm or stress was caused to the organisms involved.

Nutrient content of maggot meal

Fresh maggots were subjected to a series of meticulous preparation steps to render them into a dry and analyzable form. This involved an initial cleansing process in running water, followed by a careful drying phase at a temperature of 60°C, which spanned a duration of 24 to 48 hours. Subsequently, the dried maggots were finely ground into a powder-like consistency, facilitating further nutritional assessment. The evaluation of nutrient levels was executed employing a

comprehensive analytical approach. Firstly, the proximate method as stipulated by the Association of Official Agricultural Chemists (AOAC, 2019) was employed for the proximate analysis, providing insights into fundamental nutritional components. Additionally, the quantification of fatty acids was conducted using the sophisticated Gas Chromatography (GC) technique, which specializes in the separation and measurement of volatile compounds within the sample. Furthermore, the examination of amino acids was carried out through the application of High-Performance Liquid Chromatography (HPLC), a specialized analytical method designed for the separation and quantification of compounds within liquid samples. These analytical methodologies collectively offered a comprehensive understanding of the nutritional composition of the processed maggot material.

Production of maggot protein extract (MPE)

The extraction and preparation of the maggot-derived protein involved a series of methodical steps. Approximately 50 grams of freshly collected maggots, roughly 15 days old, were initially immersed in a phosphate buffer solution with a concentration of 100 millimolar (mM) and a pH value of 7.2. To facilitate the subsequent separation of the exoskeletal components from the valuable body fluids, the maggots were thoroughly crushed using a mortar. The body fluids, now freed from the exoskeletal remnants, underwent further refinement. They were subjected to centrifugation at 2000 revolutions per minute (rpm) at a temperature of 4°C for a duration of 10 minutes. The resulting supernatant was subsequently treated with ammonium sulfate solutions at concentrations of 70% and 80% (v/w), causing protein precipitation. Following this step, another centrifugation process was carried out at a speed of 10,000 rpm, still maintaining a temperature of 4°C, and lasting for 30 minutes. The outcome of this procedure was the collection of a protein-rich precipitate, which was then reconstituted by suspension in a phosphate buffer solution (100 mM, pH 7.2). This resulting extract was identified as Maggot Protein Extract (MPE), stemming from the larvae of the maggots, and was thoughtfully preserved in a refrigerated environment, ensuring its integrity and stability for subsequent analytical investigations.

Protein and enzyme assay

The quantification of protein concentration within the MPE was carried out employing the Bradford method, utilizing bovine serum albumin (BSA) as the reference standard protein, following the protocol outlined by (Waterborg, 2003). To determine the protein content, precisely 0.1 milliliters of the MPE sample was meticulously combined with 2.9 milliliters of the Bradford reagent. The resulting mixture was thoroughly vortexed and left to incubate for a duration of 3 minutes to facilitate the protein-reagent interaction. Subsequently, the absorbance of the prepared sample was assessed at a wavelength of 595 nanometers, yielding valuable data regarding the protein concentration within the MPE.

The determination of protease activity was conducted in accordance with the procedure outlined by Walter (in Matthews, 1987), and it was performed as follows:

1. Initially, a volume of 0.1 milliliters of Maggot Protein Extract (MPE) was mixed with 0.25 milliliters of a 1% casein solution in distinct pH buffers, specifically 50 millimolar (mM) citrate-phosphate buffers at pH 5.0 and 6.0, 50 mM Tris-Cl buffers at pH 7.0, 8.0, and 9.0, and 50 mM Borate-NaOH buffers at pH 10 and 11.0.
2. After this preparation, the mixture was incubated for a duration of 10 minutes at a temperature of 40°C.
3. To halt the enzymatic reaction, 0.5 milliliters of 0.1 M Trichloroacetic acid (5%) was introduced, followed by a subsequent incubation at 37°C for 10 minutes.
4. The resulting mixture was then subjected to centrifugation at 10,000 revolutions per minute (rpm) for a period of 5 minutes.
5. A volume of 0.75 milliliters of the supernatant was combined with 2.5 milliliters of 0.4 M Na2CO3 and 0.5 milliliters of Folin Ciocalteau reagent in a 1:4 ratio. This new mixture was incubated at 37°C for 20 minutes to induce color development.
6. Subsequently, the absorbance of the solution was measured at a wavelength of 578 nanometers.
7. For quantitative analysis, a standard curve was established using tyrosine as a reference. One unit of enzyme activity was defined as the amount of enzyme capable of liberating 1 millimole of tyrosine within a span of 1 minute, as per the generated tyrosine standard curve.

The determination of cellulase activity was executed in accordance with the methodology outlined by Camassola and J.P. Dillon (2012). The procedure is detailed as follows:

1. A circular piece of Whatman paper No. 1, measuring 25 millimeters in diameter, was positioned atop 0.25 milliliters of Maggot Protein Extract (MPE).
2. Subsequently, the setup was supplemented with 0.5 milliliters of diverse pH buffers, including 50 millimolar (mM) citrate-phosphate buffers at pH 5.0 and 6.0, 50 mM Tris-Cl buffers at pH 7.0, 8.0, and 9.0, and 50 mM Borate-NaOH buffers at pH 10 and 11.0.
3. The resulting solution was subjected to an incubation period lasting 60 minutes, maintained at a temperature of 40°C.

4. To arrest the enzymatic reactions, 1.5 milliliters of DNS (3,5-dinitrosalicylic acid) solution, prepared in a 1:4 ratio, was added.

5. The reaction was brought to a halt by boiling the reaction tube for 5 minutes and then immediately immersing it in cold water to rapidly cool it down.

6. For quantitative analysis, a standard curve was constructed using glucose as the reference. In this context, one unit of enzyme activity was defined as the amount of enzyme capable of liberating 1 millimole of glucose within a time frame of 1 minute, as determined by the generated glucose standard curve.

Statistical Analysis

For each sample, a comprehensive analysis was conducted, with every measurement carried out in triplicate to ensure the reliability of the results. The data is subsequently presented in the form of means ± standard error of the mean (SEM). The SEM, or Standard Error of the Mean, serves as a valuable indicator of the variability present within the dataset, and it is frequently employed to convey the precision associated with the calculated mean value.

RESULTS AND DISCUSSION

The Nutrient Profile of maggot meal

Protein profiles were analyzed using the NATIVE-PAGE discontinuous system on a 12% gel for separation. Protein samples were prepared by mixing protein levels and sample buffers at a ratio of 20 µl to 80 µl. Electrophoresis was carried out at a voltage of 100 volts with a constant current of 80 mA for 95 minutes. The distribution of bands was determined by staining silver-stained gels (Khoiriyah and Fatchiyah, 2013) using the Vivantis Protein Ladder (Tricolor Broad Range Prestained Protein Ladder) as a marker. The results of protein electrophoresis from three maggot sources showed the presence of distinct protein bands with estimated molecular weights of 95 kDa and 36 kDa, along with varying enzyme activity.

A thorough examination of the nutrient composition, as illustrated in Table 1, revealed discernible distinctions among three distinct types of maggots raised on varying substrates. This table presents a comprehensive overview of the nutritional content, highlighting the following key findings: Crude Protein: The maggots reared in layer manure exhibited the highest crude protein content, boasting a substantial 45.36%. In contrast, those cultivated on kitchen waste substrates displayed the lowest crude protein content, measuring in at 30.91%. Fat Content: The highest fat content, standing at 31.97%, was identified in maggots nurtured in kitchen waste. Conversely, the lowest fat content, accounting for 18.44%, was observed in maggots originating from layer manure. Crude Fiber: The analysis revealed a notably high crude fiber content, representing the cell wall components, in maggot meal sourced from larvae raised in restaurant waste (19.80%) and rejected milk substrates (19.62%). In contrast, the lowest crude fiber content, amounting to 10.14%, was derived from maggots reared on kitchen waste. Mineral Content: When considering mineral content, maggot meal obtained from kitchen waste exhibited the highest mineral concentration, reaching 21.80%. In stark contrast, the lowest mineral content, totaling 14.04%, was associated with maggot meal sourced from restaurant waste and rejected milk substrates. Nitrogen-Free Extract: The nitrogen-free extract content, which reflects carbohydrates, showcased relatively similar levels across the different maggot samples, indicating minimal variability in this particular nutritional aspect.

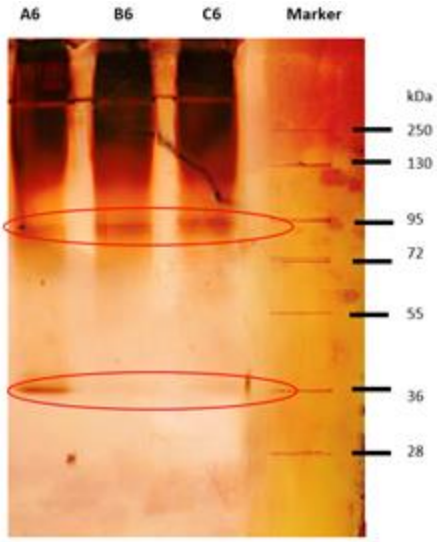

Figure 1 - Protein profile of maggot from three source of growing media (Marker contains proteins with BM 28 to 250 kDa) (A 6: Maggot Protein Concentrate (Restaurant waste and rejected milk at 60% Ammonium sulfate saturation); B 6: Maggot Protein Concentrate (Layers manure at 60% Ammonium sulfate saturation); C 6: Maggot Protein Concentrate (Kitchen waste at 60% Ammonium sulfate saturation) (C))

Table 1 - The nutrient content of maggot meal from rearing various substrates

Nutrient \ Maggot substrates	Restaurant waste and rejected milk (A)	Layers manure (B)	Kitchen waste (C)
Dry Matter (%)	96.11 ± 0.05	95.37 ± 0.06	95.38 ± 0.11
Crude Protein (%)	43.33 ± 0.08	45.36 ± 0.25	30.91 ± 0.63
Crude Fat (%)	22.29 ± 0.06	18.44 ± 0.11	31.97 ± 0.35
Crude Fiber (%)	19.80 ± 0.14	19.62 ± 0.11	10.14 ± 4.97
Ash (%)	14.04 ± 0.03	16.15 ± 0,.14	21.80 ± 0.71
NFE (%)	0.54 ± 0.10	0.42 ± 0.12	0.56 ± 0.11

NFE= Nitrogen-Free Extract: A measure of the carbohydrate content in a sample.

Variations in protein content and other nutrients closely reflect the nutrient availability of maggot-growing substrates. The layer manure substrates showed a high level of consistency compared to the others, which was attributed to the fact that the nutrient composition of commercial layer feed did not change during maintenance. The feed conversion ability of maggots is highly dependent on the availability of nutrient-rich substrates for growth. As elucidated by Gobbi et al. (2013) and Makkar et al. (2014), it has been established that the characteristics of the rearing medium play a pivotal role in determining both the nutritional quality and quantity of the body constituents in fly larvae at various developmental stages. Furthermore, these factors exert a considerable influence on larval survival during each instar and their subsequent progression through the metamorphic stages. Discrepancies observed in the nutrient content of rearing substrates, with particular attention to variations in carbohydrate and protein concentrations and their interplay, have the potential to induce notable adaptations in the behavior and physiological responses of insects (Simpson et al., 2015). The adaptability of the midgut in *H. illucens* larvae enables them to thrive on a multitude of feeding substrates by effectively adjusting to varying nutrient compositions (Bonelli et al., 2020). The same patterns of amino acid levels were observed in three types of maggot meal derived from larvae grown in different sources of media. In this investigation, a set of vital amino acids, encompassing valine, leucine, isoleucine, phenylalanine, methionine, threonine, histidine, lysine, and arginine, were discerned and acknowledged. Conversely, among the amino acids analyzed, aspartic acid, glutamic acid, serine, alanine, and tyrosine were identified as non-essential. The six amino acids with the highest levels in maggot flour were glutamate, aspartate, alanine, valine, leucine, and isoleucine. Meanwhile, the highest essential amino acid was leucine, and the lowest was methionine.

Essential branched-chain amino acids, commonly referred to as BCAAs, are a subgroup of indispensable amino acids, specifically isoleucine, leucine, and valine, that play a pivotal role in various biological processes, particularly in the context of livestock nutrition. These BCAAs are noteworthy for their unique characteristics, including being synthesized primarily within muscle tissues, a process integral to their function. One of their prominent functions in livestock is to act as safeguards against muscle tissue damage. When animals engage in activities that can exert significant stress on their muscles, such as physical exercise or growth, these BCAAs are essential in preventing the breakdown of muscle tissue. They do so by serving as a readily available energy source during periods of heightened demand, reducing the need for the body to break down muscle protein for energy. In essence, BCAAs act as a protective barrier, preserving the structural integrity of muscle tissue and preventing unwarranted muscle loss.

Table 2 - Amino acids of maggot meal rearing on various substrates

Maggot substrates / Amino acid (%)	Restaurant waste and rejected milk (A)	Layers manure (B)	Kitchen waste (C)
Aspartic acid	3.35	3.29	3.57
Glutamic acid	4.56	3.5	11.50
Serine	1.40	1.59	1.50
Histidine	0.66	0.82	1.00
Glycine	1.82	3.49	1.80
Threonine	1.72	1.38	1.43
Arginine	1.90	1.56	1.76
Alanine	2.37	2.43	3.11
Tyrosine	2.02	2.17	2.00
Methionine	0.26	0.13	0.05
Valine	2.29	2.40	2.46
Pheylalanine	1.57	1.52	1.65
l-Leucine	1.74	1.72	2.03
Leucine	2.56	2.56	2.65
Lysine	1.86	1.66	1.55
Total Amino Acid	30.07	30.20	38.06

Additionally, BCAAs are utilized to balance hormone release and brain function (Chasanah et al., 2015). The highest non-essential amino acid is glutamic acid, which, according to Winarno (2004) it was determined that the most abundant among the non-essential amino acids is glutamic acid. This particular amino acid has been noted for its significant role in supporting and enhancing various aspects of brain function. Notably, glutamic acid is associated with the facilitation of learning processes and the enhancement of memory, underscoring its importance in cognitive functioning. Glutamic acid also promotes an increase in muscle mass. Both glutamic acid and glutamine are interconvertible (Dutta et al., 2013), while among the non-essential amino acids, only glutamic acid is classified within the major group of neurotransmitters (Shih et al., 2005). Glutamic acid serves as a conjugate due to its capacity to enhance the effectiveness of an anti-cancer

medication while concurrently reducing its harmful impact on healthy cells. Dutta et al. (2013) highlighted the critical role of glutamine in maintaining optimal immune functionality. They emphasized that glutamine is essential for supporting the proliferation of lymphocytes and cytokines, crucial elements in immune system regulation. Furthermore, glutamine enhances the effectiveness of macrophages, which are substantial immune cells responsible for the ingestion and breakdown of various foreign materials, encompassing microbes and even inorganic compounds.

Elwert et al. (2010) compared the amino acid patterns of fish meals with reduced-fat BSF meal (BSF-37) and reported a relatively similar pattern. Through an examination of amino acid profiles relative to lysine, it was apparent that the levels of isoleucine, leucine, threonine, valine, phenylalanine, and arginine were notably more abundant in black soldier fly (BSF) meal when compared to fish meal. However, a significant contrast was noted in the histidine content, with black soldier fly (BSF) flour exhibiting a relatively lower concentration of methionine in comparison to that found in fish meals. This difference in amino acid composition underscores the distinct nutritional attributes of BSF meal in relation to its histidine and methionine content when contrasted with traditional fish meal.

Table 3 - Fatty acids of maggot meal rearing on various substrates

Maggot substrates / Fatty Acid (%/%, w/w)	Restaurant waste and rejected milk (A)	Layers manure (B)	Kitchen waste (C)
Butyric acid	-	0.05	-
Caproic acid, C6:0	0.18	-	-
Caprilic acid, C8:0	0.65	0.07	-
Capric acid, C10:0	0.47	0.90	0.58
Lauric acid, C12:0	22.94	32.18	33.79
Myristic acid, C14:0	5.4	4.56	7.73
Myristoleic acid, C14:1	0.05	0.06	0.12
Tridecanoic acid, C 13:0	-	-	0.06
Pentadecanoic acid, C15:0	0.11	0.28	0.52
Palmitic acid, C16:0	19.70	9.68	13.92
Palmitoleic acid, C16:1	1.16	3.11	-
Heptadecanoic acid, C17:0	0.08	0.17	0.21
Cis-10-Heptadecanoic acid, C17:1	0.05	0.18	0.32
Stearic acid, C18:0	1.76	1.39	2.47
Elaidic acid, C18:1, n9c	0.15	0.42	0.14
Oleic acid, C18:1, n9c	12.58	15.13	10.33
Linoleic acid, C18:2, n6c	0.09	0.05	6.23
Arachidic acid: C20:2	0.35	8.35	0.07
Gama-linolenic C18:3, n6	0.09	0.06	0.11
Linolenic acid C18:3, n3	0.03	-	0.51
Heneicosanoic acid, C21:0	0.23	0.15	-
Cis 11,14 Eicosedienoic acid, C20:2	0.15	0.03	0.13
Cis-11-Eicosenoic acid, C20:1	-	-	0.16
Behenic acid, C22:0	0.03	-	-
Cis 11, 14, 17-Eicosatrienoic, C20:3, n6	0.02	0.02	0.20
Cis-5,8,11,14,17-Eicosapentaenoic acid, C20:5, n3	-	-	0.38
Cis-4,710,13,16,19-Docosahexaenoic acid, C22:6, n3	-	-	0.13
Arachidonic acid, C20:4, n6	-	0.17	0.40
Lignoceric acid C24:0	-	0.01	0.02
Nervonic acid (C24:1, n-9)	0.02	0.09	-
Total Fatty acid	66.30	77.13	-

As illustrated in Table 3, an in-depth analysis of the composition of major fatty acids within maggot meal derived from a range of substrates unveils several notable components. These prominent fatty acids encompass lauric acid (C12:0), known for its distinct characteristics, as well as palmitic acid (C16:0), oleic acid (C18:1), palmitoleic acid (C20:1), and stearic acid (C18:0), each playing a distinct role in the overall lipid profile of the maggot meal. Linoleic acid (C18:2) was only found in maggot meal from kitchen waste (C), while arachidic acid (C20:2) was observed only in maggot meal from layer manure (B). It's apparent that lauric acid stands out as the predominant fatty acid present in all three variations of maggots cultivated on diverse substrates. The lowest concentration of lauric acid was observed in maggot meal from restaurant waste and rejected milk (A), while the highest was in maggot meal from kitchen waste (C).

Apart from the presence of medium-chain saturated fatty acids, it's worth noting that maggot oil also exhibited relatively high concentrations of unsaturated fatty acids, particularly oleic acid. The percentage of oleic acid in maggot oil

ranged from 10.33% to 15.13%, underscoring the diversity of fatty acid components within this oil. The highest concentration of oleic acid was found in maggot meal derived from layer manure (B), making it a potentially better source of fatty acids than others. It is noteworthy to recognize that the fatty acid content within the larvae is not solely an intrinsic characteristic but, rather, is subject to partial modulation by the specific fatty acid composition present in the substrate on which these larvae are nurtured. This interaction between the dietary source and the resultant fatty acid content in the larvae underscores the complex and interconnected relationship between the nutritional environment and the composition of the organisms that develop within it. Lauric acid may also exhibit potential therapeutic properties to boost the body's immune system. When enzymes in the body hydrolyze lauric acids, they produce a bioactive compound known as monolaurin. Monolaurin has been the subject of scientific studies for its potential antifungal, antibacterial, and antiviral properties, suggesting that maggots could be a valuable source of antimicrobial agents. Numerous studies conducted by various researchers have explored fatty acids encompassing carbon chains ranging from 6 to 18 carbons, as well as select derivatives featuring diverse functionalized headgroups. Through this extensive investigation, it was conclusively determined that lauric acid (LA) with the molecular notation C12:0 emerged as the most potent antimicrobial lipid, effectively impeding the growth of Gram-positive bacteria (Schlievert et al., 1992; Subroto and Indiarto, 2020; Yoon et al., 2018).

Oleic acid, known as omega-9 fatty acids, is not an essential fatty acid and can be synthesized in the human body from stearic acid through a reaction catalyzed by D9-desaturase, unlike omega-3 and omega-6 fatty acids (Delgado et al., 2017). Studies by Schwingshackl and Hoffmann (2014) reported that dietary monounsaturated fatty acids, including oleic, gondoic, and nervonic acid, reduced the overall risk of all-cause mortality (11%), cardiovascular mortality (12%), cardiovascular events (9%), and stroke (9%).

Protein concentration and activity of protease and cellulase of MPE

The Bradford protein assay finds application in the quantification of total protein concentration within a given sample. The fundamental principle governing this assay is based on the interaction between protein molecules and Coomassie dye in an acidic environment, leading to a discernible color transition from brown to blue. The protein concentration of MPE ranged from 0.56 mg/ml to 0.601 mg/ml under 60% (w/v) ammonium sulfate precipitation, and from 0.555 mg/ml to 0.609 mg/ml under 70% (w/v) ammonium sulfate precipitation. The concentration of MPE protein appeared to be relatively consistent between both 60% and 70% ammonium sulfate precipitation. However, MPE derived from restaurant waste and rejected milk exhibited the lowest protein concentration.

Table 4 - Protein concentration of mpe from various source

Protein (mg/ml) / Substrates	60% (w/v) ammonium sulphate saturation	70% (w/v) ammonium sulphate saturation
Restaurant waste and rejected milk (A)	0.560 ± 0.0009	0.555 ± 0.0014
Layers manure (B)	0.601 ± 0.0185	0.609 ± 0.0002
Kitchen waste (C)	0.601 ± 0.0023	0.609 ± 0.0173

Protease, an enzyme with extensive applications, is commonly harnessed within the food industry to expedite the hydrolysis of peptide bonds present in protein molecules. This enzymatic action contributes to the enhancement of product quality and augments the nutraceutical value (Palsaniya et al., 2012). The diversity of proteases has implications for the molecular weight (MW or Molecular Weight: The average mass of molecules in a sample) and the amino acid sequence of the peptides they produce, resulting in different biological activities. The digestive system of *Hermetia illucens* larvae is equipped with protease enzymes, which empower them to effectively break down a wide range of organic materials and subsequently convert them into valuable protein resources (Kim et al., 2011).

The microbiome residing within the gut of Black Soldier Fly Larvae (BSFL) and the external bacterial communities associated with them demonstrate the capacity to generate and release microbial enzymes, including proteases, cellulases, lipases, xylanases, and pectinases. These enzymes play a crucial role in the breakdown of organic compounds found in animal manure. It seems that the majority of proteins and other organic substances within animal waste are degraded through the action of these digestive enzymes. These findings imply that the richness and diversity of microorganisms in animal waste play a substantial role in influencing the BSFL's proficiency in decomposing various types of organic waste (De Smet et al., 2018).

Measuring protease activity at various pH and a temperature of 40°C produced the following results. The optimum protease activity of MPE obtained from restaurant waste and rejected milk (A) was shown at pH 5.0, both at 60% and 70% ammonium sulfate precipitation, namely 0.451 U/mg and 0.656 U/mg, respectively. The optimum protease activity

of MPE (0.748 U/mg) from layer manure was observed at pH 7.0 (precipitation of 60% ammonium sulfate), and at 70% deposition of ammonium sulfate, the optimum activity was shown at pH 11.0, namely 0.774 U/mg. Meanwhile, the activity of protease MPE from kitchen waste showed optimal activity at pH 10.0 at either 60% or 70% ammonium sulfate precipitation, namely 0.617 and 0.821 U/mg, respectively (Figure 2).

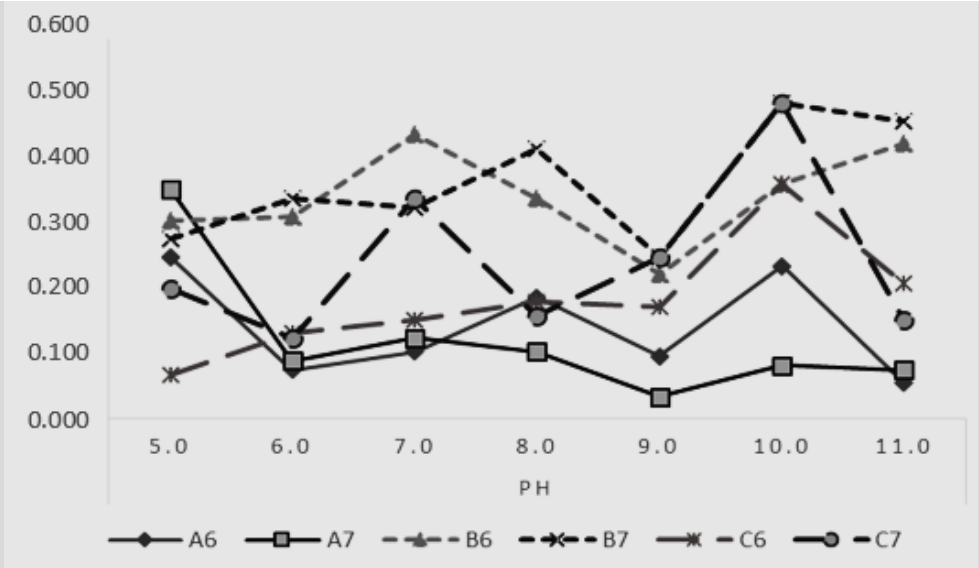

Figure 2 - Protease Activity of Maggot Protein Extract (MPE) From Various Substrates on various pH at 40° C. (A, MPE from restaurant waste and rejected milk; B, MPE from layer manure; C, MPE from kitchen waste; numbers 6 and 7 refer to 60% and 70% ammonium precipitation).

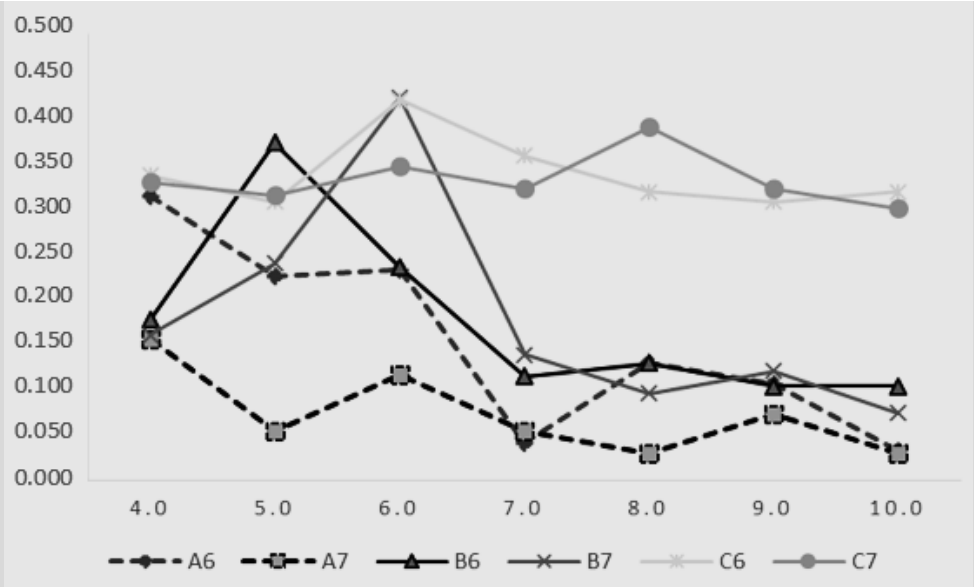

Figure 3 - Cellulase Activity of Maggot Protein Extract (MPE) From Various Substrates on various pH at 40° C. (A, MPE from restaurant waste and rejected milk; B, MPE from layer manure; C, MPE from kitchen waste; numbers 6 and 7 refer to 60% and 70% ammonium precipitation).

Supriyatna andUkit (2015) reported that the activity of protease enzymes from intestinal extracts of *H. illucens* larvae grown on rice straw media and incubated at various temperatures of 30°C, 35°C, 40°C, 45°C, and 50°C were 0.57 U/ml, 0.67 U/ml, 0.87 U/ml, 0.96 U/ml, 0.77 U/ml, respectively. At 45°C, the protease enzyme reached the optimum temperature to hydrolyze the substrate with an activity value of 0.96 U/ml. The difference in protease activity in this study was attributed to the sampling process, which involved extracting not only the digestive tract (gut) but the entire internal organs of the whole body. The optimal activity of protease tended to be acidic in maggots grown in restaurant waste and rejected milk (A), neutral in layer manure (B), and basic in kitchen waste (C). Digestive enzymes in maggot extract are mainly derived from the salivary glands and gut (Kim et al., 2011). Therefore, the fact that MPE contains various types of proteases from the digestive tract with various properties is comprehensible. Bonelli et al. (2019) serine proteases, which

are endopeptidases optimized for alkaline pH conditions, assume a primary role in the initial stage of protein breakdown within the posterior segment of the black soldier fly (BSF) larval midgut. Insects commonly feature two primary serine proteases, known as trypsin and chymotrypsin (Walter R. Terra and Ferreira, 1994; Walter Ribeiro Terra et al., 1996; Bonelli et al., 2020) found in the posterior midgut of larvae (Bonelli et al., 2020). Lysozyme is an acidic protease generated within the luminal environment of the anterior and middle segments of the midgut tract. It potentially serves a significant function in eradicating pathogenic microorganisms that may be introduced into the digestive system through the ingested feeding substrate (Bonelli et al., 2019).

Maggot protein extract (MPE) obtained from various substrates exhibited cellulolytic activity, with the highest activity observed in kitchen waste substrates (C) at both 60% and 70% ammonium sulfate (Figure 3). While cellulase from C substrates appeared stable over a wide pH range of 5.0-11.0, that from B substrates (layer manure) was only optimal at pH 5.0 (60% ammonium sulfate) and 6.0 (70% ammonium sulfate). The lowest cellulase optimum activity was clearly observed in A substrates (restaurant waste and rejected milk) at pH 4.0 and 5.0. These varied results support the notion that rearing substrates affect cellulase activity and that kitchen waste contains higher amounts of crude fiber than layer manure, restaurant waste, and rejected milk.

The gut of Black Soldier Fly (BSF) larvae serves as a thriving ecosystem for a diverse array of cellulolytic bacteria, with cellulase production being primarily attributed to genera like *Bacillus* sp., *Bacillus thuringiensis*, *Ruminococcus* sp., and *Proteus* sp., (Supriyatna and Ukit, 2015). Among these, the *Ruminococcus* genus stands out, particularly certain species such as *R. albus* and *R. flavefaciens*, which exhibit the remarkable capability to ferment highly structured cellulose fibers and generate cellulase (Shweta, 2012).

Bacillus sp., a well-known genus of bacteria, is renowned for its capacity to secrete a wide spectrum of enzymes, including cellulolytic enzymes. The cellulases produced by Bacillus species are predominantly extracellular and soluble in nature. Notable strains within the Bacillus genus that are recognized for their cellulase secretion encompass *B. subtilis*, *B. polymyxa*, *B. licheniformis*, and *B. cereus*. This multifaceted enzymatic activity further underscores the significance of these cellulolytic microorganisms within the BSF larval gut environment.

CONCLUSION

In conclusion, this study investigated the nutrient profiles, protease, and cellulase activities of maggot protein extract (MPE) from *Hermetia illucens* larvae reared on different substrates. The key findings are as follows:

1. Nutrient profiles: The nutrient composition of maggot meal varied significantly depending on the rearing substrate. Maggot meal from layer manure had the highest protein content (45.36%) and the lowest fat content (18.44%). Amino acid profiles also differed among substrates, with maggot meal from kitchen waste containing high levels of glutamic acid and maggot meal from restaurant waste and rejected milk having notable lauric acid content.
2. Protein concentration: The protein concentration of MPE from different substrates showed a relatively consistent range, ranging from 0.555 mg/ml to 0.609 mg/ml, with layer manure-based MPE exhibiting the highest concentration.
3. Protease activity: The protease activity of MPE varied with pH and substrate source. MPE from layer manure had the highest protease activity at pH 7.0 and pH 11.0, while MPE from restaurant waste and rejected milk had its optimum activity at pH 5.0.
4. Cellulase activity: Kitchen waste-based MPE displayed the highest cellulase activity and remained stable over a wide pH range (pH 5.0-11.0), while MPE from other substrates showed varying pH optima for cellulase activity.

Suggestion

Future research in this field should prioritize several key areas. Firstly, the optimization of rearing substrates for maggot cultivation must be explored to identify the most suitable substrates for specific applications. A more detailed characterization of protease and cellulase enzymes in maggot protein extract (MPE) should be conducted to unlock their industrial potential. The development of innovative products and supplements using maggot-derived nutrients and enzymes should be explored for applications in animal feed and more. Investigating the role of *Hermetia illucens* larvae in waste decomposition and waste management systems is a promising avenue. Lastly, comprehensive safety and nutritional assessments of maggot-derived products are crucial for potential use in both animal and human diets, contributing to more sustainable food and waste management solutions.

DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Titin Widiyastuti; E-mail: titin.widiyastuti@unsoed.ac.id; ORCID: https://orcid.org/0000-0003-0033-816X

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics committee approval

This study was approved by the Directorate General of Higher Education, Ministry of Education and Culture of the Republic of Indonesia in 2022, under Decision Letter (SK) number 2127/UN23/PT.01.02/2022.

Authors contribution

T. Widiyastuti was responsible for data collection, statistical analysis, contributing to result interpretation, and drafting the initial manuscript. S. Rahayu was the driving force behind formulating the original hypotheses, experiment design, result interpretation, and manuscript finalization. W. Suryapratama and F. M. Suhartati, both authors, have reviewed and given their approval for the completed manuscript.

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Consent for publication

All participants have consented to the submission of the review article to the journal.

Competing interests

The authors declare no competing interests in this research and publication.

REFERENCES

Abduh, M. Y., Perdana, M. P., Bara, M. A., Anggraeni, L. W., & Putra, R. E. (2022). Effects of aeration rate and feed on growth, productivity and nutrient composition of black soldier fly (*Hermetia illucens* L.) larvae. *Journal of Asia-Pacific Entomology*, *25*(2), 101902. https://doi.org/10.1016/j.aspen.2022.101902

AOAC. (2019). *Official methods of analysis, Association of official analytical chemist 21st edition*. Association of Official Analytical Chemists. https://www.scirp.org/reference/referencespapers?referenceid=2939092

Arief, M., Ratika, A. N., & Lamid, M. (2012). Pengaruh kombinasi media bungkil kelapa sawit dan dedak padi yang difermentasi terhadap produksi maggot black soldier fly (Hermetia illucens) sebagai sumber protein pakan ikan [The effect of a combination of fermented palm kernel cake and rice bran media on the production of black soldier fly maggots (Hermetia illucens) as a source of fish feed protein]. *Jurnal Ilmiah Perikanan Dan Kelautan*, *4*(1), 33–37. https://doi.org/10.20473/jipk.v4i1.11580

Banks, I. J., Gibson, W. T., & Cameron, M. M. (2014). Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. *Tropical Medicine and International Health*, *19*(1), 14–22. https://doi.org/10.1111/tmi.12228

Bonelli, M., Bruno, D., Brilli, M., Gianfranceschi, N., Tian, L., Tettamanti, G., Caccia, S., & Casartelli, M. (2020). Black soldier fly larvae adapt to different food substrates through morphological and functional responses of the midgut. *International Journal of Molecular Sciences*, *21*(14), 4955. https://doi.org/10.3390/ijms21144955

Bonelli, M., Bruno, D., Caccia, S., Sgambetterra, G., Cappellozza, S., Jucker, C., Tettamanti, G., & Casartelli, M. (2019). Structural and functional characterization of hermetia illucens larval midgut. *Frontiers in Physiology*, *10*, 427234. https://doi.org/10.3389/fphys.2019.00204

Bosch, G., Zhang, S., Oonincx, D. G. A. B., & Hendriks, W. H. (2014). Protein quality of insects as potential ingredients for dog and cat foods. *Journal of Nutritional Science*, *3*, e29. https://doi.org/10.1017/jns.2014.23

Camassola, M., & J.P. Dillon, A. (2012). Cellulase determination: modifications to make the filter paper assay easy, fast, practical and efficient. *Journal of Analytical & Bioanalytical Techniques*, *01*(S1), 25. https://doi.org/10.4172/scientificreports.125

Chasanah, E., Nurilmala, M., Purnamasari, A. R., & Fithriani, D. (2015). Chemical composition, albumin content and bioactivity of crude protein extract of native and cultured channa striata. *Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan*, *10*(2), 124–132. https://www.academia.edu/31670751/Chemical_Composition_Albumin_Content_and_Bioactivity_of_Crude_Protein_Extract_of_Native_and_Cultured_Channa_striata

De Smet, J., Wynants, E., Cos, P., & Van Campenhout, L. (2018). Microbial community dynamics during rearing of black soldier fly larvae (Hermetia illucens) and impact on exploitation potential. *Applied and Environmental Microbiology*, *84*(9), e02722-17. https://doi.org/10.1128/AEM.02722-17

Delgado, G. E., Krämer, B. K., Lorkowski, S., März, W., von Schacky, C., & Kleber, M. E. (2017). Individual omega-9 monounsaturated fatty acids and mortality—The Ludwigshafen Risk and Cardiovascular Health Study. *Journal of Clinical Lipidology*, *11*(1), 126-135. e5. https://doi.org/10.1016/j.jacl.2016.10.015

Dengah, S. P., Umboh, J. F., Rahasia, C. A., & Kowel, Y. H. . (2015). Pengaruh penggantian tepung ikan dengan tepung maggot (hermetia illucens) dalam ransum terhadap performans broile [The effect of replacing fish meal with maggot meal (Hermetia illucens) in the diet on broiler performance]. *Zootec*, *36*(1), 51–60. https://doi.org/10.35792/zot.36.1.2016.9444

Dong, S., Chen, Y., Huang, Y., & Feng, D. (2009). Research on feed characteristics of Bacillus Natto. *Chinese Journal of Animal Nutrition*, *21*(3), 371–378. https://www.cabidigitallibrary.org/doi/full/10.5555/20093194519

Dordević, M., Brana, R. D., Marijana, V., Baltić, M., Radislava, T., Ljiljana, J., Marija, V., & Rajković, M. (2008). Effects of substitution of fish

meal with fresh and dehydrated larvae of the house fly (*Musca domestica* L) on productive performance and health of broilers. *Acta Veterinaria*, *58*(4), 357–368. https://doi.org/10.2298/AVB0804357D

Dossey, A. T., Tatum, J. T., & McGill, W. L. (2016). Modern insect-based food industry: current status, insect processing technology, and recommendations moving forward. In *Insects as Sustainable Food Ingredients: Production, Processing and Food Applications* (pp. 113–152). https://doi.org/10.1016/B978-0-12-802856-8.00005-3

Dutta, S., Ray, S., & Nagarajan, K. (2013). Glutamic acid as anticancer agent: An overview. *Saudi Pharmaceutical Journal*, *21*(4), 337–343. https://doi.org/10.1016/j.jsps.2012.12.007

Elwert, C., Knips, I., & Katz, P. (2010). A novel protein source: Maggot meal of the Black Soldier fly (Hermetia illucens) in broiler feed. *11. Tagung Schweine- Und Geflügelernährung*, *November 2010*, 40–142. https://www.feedtest.de/Publikationen/2010 SGE Hermetia meal.pdf

Gobbi, P., Martínez-Sánchez, A., & Rojo, S. (2013). The effects of larval diet on adult life-history traits of the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). *European Journal of Entomology*, *110*(3), 461–468. https://doi.org/10.14411/eje.2013.061

Gold, M., Tomberlin, J. K., Diener, S., Zurbrügg, C., & Mathys, A. (2018). Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: A review. *Waste Management*, *82*, 302–318. https://doi.org/10.1016/j.wasman.2018.10.022

Green, T. R., & Popa, R. (2012). Enhanced ammonia content in compost leachate processed by black soldier fly larvae. *Applied Biochemistry and Biotechnology*, *166*(6), 1381–1387. https://doi.org/10.1007/s12010-011-9530-6

Hahn, T., Roth, A., Febel, E., Fijalkowska, M., Schmitt, E., Arsiwalla, T., & Zibek, S. (2018). New methods for high-accuracy insect chitin measurement. *Journal of the Science of Food and Agriculture*, *98*(13), 5069–5073. https://doi.org/10.1002/jsfa.9044

Katayane, F. A., Bagau, B., Wolayan, F. R., & Imbar, M. R. (2014). Produksi dan kandungan protein maggot (Hermetia illucens) dengan menggunakan media tumbuh berbeda[Production and protein content of maggots (Hermetia illucens) using different growth media]. *Zootec*, *34*, 27–36. https://ejournal.unsrat.ac.id/index.php/zootek/article/view/4791

Khoiriyah, L. K., & Fatchiyah, F. (2013). Karakter biokimia dan profil protein yogurt kambing pe difermentasi bakteri asam laktat (BAL). *The Journal of Experimental Life Science*, *3*(1), 1–6. https://doi.org/10.21776/ub.jels.2013.003.01.01

Kim, W., Bae, S., Park, K., Lee, S., Choi, Y., Han, S., & Koh, Y. (2011). Biochemical characterization of digestive enzymes in the black soldier fly, Hermetia illucens (Diptera: Stratiomyidae). *Journal of Asia-Pacific Entomology*, *14*(1), 11–14. https://doi.org/10.1016/j.aspen.2010.11.003

Lalander, C., Diener, S., Magri, M. E., Zurbrügg, C., Lindström, A., & Vinnerås, B. (2013). Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens) - From a hygiene aspect. *Science of the Total Environment*, *458*, 312–318. https://doi.org/10.1016/j.scitotenv.2013.04.033

Liu, C., Wang, C., Yao, H., & Chapman, S. J. (2021). Pretreatment is an important method for increasing the conversion efficiency of rice straw by black soldier fly larvae based on the function of gut microorganisms. *Science of the Total Environment*, *762*, 144118. https://doi.org/10.1016/j.scitotenv.2020.144118

Makkar, H. P. S., Tran, G., Heuzé, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. In *Animal Feed Science and Technology* (Vol. 197, pp. 1–33). https://doi.org/10.1016/j.anifeedsci.2014.07.008

Matthews, R. (1987). Methods of enzymatic analysis. *Journal of Clinical Pathology*, *40*(8). https://doi.org/10.1136/jcp.40.8.934-a

Maulana, M., Nurmeiliasari, N., & Fenita, Y. (2021). Pengaruh Media Tumbuh yang Berbeda terhadap Kandungan Air, Protein dan Lemak Maggot Black Soldier Fly (Hermetia illucens). *Buletin Peternakan Tropis*, *2*(2), 149–157. https://doi.org/10.31186/bpt.2.2.149-157

Moula, N., & Detilleux, J. (2019). A meta-analysis of the effects of insects in feed on poultry growth performances. *Animals*, *9*(5). https://doi.org/10.3390/ani9050201

Palsaniya, P., Mishra, R., Beejawat, N., Sethi, S., & Gupta, B. L. (2012). Optimization of alkaline protease production from Bacteria isolated from soil. *Journal of Microbiology and Biotechnology Research*, *2*(6), 858–865. https://scholar.google.com/scholar?cluster=12320151028161225441&hl=en&as_sdt=0,5

Rambet, V., Umboh, J. F., Tulung, Y. L. R., & Kowel, Y. H. S. (2015). Kecernaan protein dan energi ransum broiler yang menggunakan tepung maggot (hermetia illucens) sebagai pengganti tepung ikan[Protein and energy digestibility of broiler rations using maggot meal (Hermetia illucens) as a replacement for fish meal]. *Zootec*, *35*(2), 13–22. https://doi.org/10.35792/zot.36.1.2016.9314

Rinaudo, M. (2006). Chitin and chitosan: properties and applications. *Progress in Polymer Science (Oxford)*, *31*(7), 603–632. https://doi.org/10.1016/j.progpolymsci.2006.06.001

Schlievert, P. M., Deringer, J. R., Kim, M. H., Projan, S. J., & Novick, R. P. (1992). Effect of glycerol monolaurate on bacterial growth and toxin production. *Antimicrobial Agents and Chemotherapy*, *36*(3), 626–631. https://doi.org/10.1128/AAC.36.3.626

Schwingshackl, L., & Hoffmann, G. (2014). Monounsaturated fatty acids, olive oil and health status: A systematic review and meta-analysis of cohort studies. *Lipids in Health and Disease*, *13*(1), 1–15. https://doi.org/10.1186/1476-511X-13-154

Shih, I.-, Van, Y.-, & Shen, M.-. (2005). Biomedical applications of chemically and microbiologically synthesized poly(glutamic acid) and poly(lysine). *Mini-Reviews in Medicinal Chemistry*, *4*(2), 179–188. https://doi.org/10.2174/1389557043487420

Shumo, M., Osuga, I. M., Khamis, F. M., Tanga, C. M., Fiaboe, K. K. M., Subramanian, S., Ekesi, S., van Huis, A., & Borgemeister, C. (2019). The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. *Scientific Reports*, *9*(1), 10110. https://doi.org/10.1038/s41598-019-46603-z

Shweta, A. (2012). Cellulases of bacterial origin and their applications: A review. *International Journal of Science and Research*, *3*(10), 1652. https://scholar.google.com/scholar?cluster=3138718175972059029&hl=en&as_sdt=0,5

Simpson, S. J., Le Couteur, D. G., & Raubenheimer, D. (2015). Putting the balance back in diet. *Cell*, *161*(1), 18–23. https://doi.org/10.1016/j.cell.2015.02.033

Soetemans, L., Uyttebroek, M., & Bastiaens, L. (2020). Characteristics of chitin extracted from black soldier fly in different life stages. *International Journal of Biological Macromolecules*, *165*, 3206–3214. https://doi.org/10.1016/j.ijbiomac.2020.11.041

Spranghers, T., Ottoboni, M., Klootwijk, C., Ovyn, A., Deboosere, S., De Meulenaer, B., Michiels, J., Eeckhout, M., De Clercq, P., & De Smet, S. (2017). Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. Journal

of the Science of Food and Agriculture, *97*(8), 2594–2600. https://doi.org/10.1002/jsfa.8081

Subroto, E., & Indiarto, R. (2020). Bioactive monolaurin as an antimicrobial and its potential to improve the immune system and against covid-19: A review. Food Research, *4*(6), 2355–2365. https://doi.org/10.26656/fr.2017.4(6):324

Suciati, R., & Faruq, H. (2017). Efektivitas media pertumbuhan maggot Hermetia illucens (lalat tentara hitam) sebagai solusi pemanfaatan sampah organik [Effectiveness of maggots (Hermetia illucens) growth media (black soldier fly) as a solution for organic waste utilization]. BIOSFER : Jurnal Biologi Dan Pendidikan Biologi, *2*(1): 8–13. https://doi.org/10.23969/biosfer.v2i1.356

Supriyatna, A., & Ukit, U. (2015). Aktivitas enzim amilase, lipase, dan protease dari larva Hermetia illucens yang diberi pakan jerami padi [Amylase, lipase, and protease enzyme activities of Hermetia illucens larvae fed with rice straw]. Jurnal Istek, *9*(2), 18–32. https://core.ac.uk/download/pdf/234030869.pdf

Terra, Walter R., & Ferreira, C. (1994). Insect digestive enzymes: properties, compartmentalization and function. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, *109*(1), 1–62. https://doi.org/10.1016/0305-0491(94)90141-4

Terra, Walter Ribeiro, Ferreira, C., Jordao, B. P., & Dillon, R. J. (1996). Digestive enzymes. In *Biology of the insect midgut* (pp. 153–194). Springer. https://doi.org/10.1007/978-94-009-1519-0_6

Tschirner, M., & Simon, A. (2015). Influence of different growing substrates and processing on the nutrient composition of black soldier fly larvae destined for animal feed. Journal of Insects as Food and Feed, *1*(4), 249–259. https://doi.org/10.3920/JIFF2014.0008

van Huis, A. (2020). Insects as food and feed, a new emerging agricultural sector: A review. Journal of Insects as Food and Feed, *6*(1), 27–44. https://doi.org/10.3920/JIFF2019.0017

Wang, H., Rehman, K. U., Liu, X., Yang, Q., Zheng, L., Li, W., Cai, M., Li, Q., Zhang, J., & Yu, Z. (2017). Insect biorefinery: A green approach for conversion of crop residues into biodiesel and protein. Biotechnology for Biofuels, *10*(1), 1–13. https://doi.org/10.1186/s13068-017-0986-7

Wang, Y. S., & Shelomi, M. (2017). Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. Foods, *6*(10), 91. https://doi.org/10.3390/foods6100091

Waterborg, J. H. (2003). The Lowry Method for Protein Quantitation. In The Protein Protocols Handbook. https://doi.org/10.1385/1-59259-169-8:7

Widjastuti, T., Wiradimadja, R., & Rusmana, D. (2014). The Effect of Substitution of Fish Meal by Black Soldier Fly (Hermetia illucens) Maggot Meal in the Diet on Production Performance of Quail (Coturnix coturnix japonica). Scientific Papers. *Series D. Animal Science*, *LVII*, 125–129. https://animalsciencejournal.usamv.ro/pdf/2014/art21.pdf

Winarno, F. G. (2004). *Kimia pangan dan gizi* (Vol. 13, Issue 2). Gramedia Pustaka Utama.

Yoon, B. K., Jackman, J. A., Valle-González, E. R., & Cho, N. J. (2018). Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing, and therapeutic applications. International Journal of Molecular Sciences, *19*(4), 1114. https://doi.org/10.3390/ijms19041114

Yu, G., Cheng, P., Chen, Y., Li, Y., Yang, Z., Chen, Y., & Tomberlin, J. K. (2011). Inoculating poultry manure with companion bacteria influences growth and development of black soldier fly (Diptera: Stratiomyidae) larvae. Environmental Entomology, *40*(1), 3–35. https://doi.org/10.1603/EN10126

Zheng, L., Hou, Y., Li, W., Yang, S., Li, Q., & Yu, Z. (2012). Biodiesel production from rice straw and restaurant waste employing black soldier fly assisted by microbes. Energy, *47*(1), 125–129. https://doi.org/10.1016/j.energy.2012.09.006