Thesis progression

Thesis title: The production of antimicrobial peptides from black soldier fly larvae (*Hermetia illucens*) against chicken bacterial pathogens

Thesis progression title: Immunization of BSF larvae and characterization of crude AMP: Protein quantification and antimicrobial activity

Student: Miss Prawphan Kotthale **Student ID:** 677070004-1

Advisor: Assist. Prof. Dr. Umaporn Yordpratum

Co-Advisor: Asst. Prof. Dr. Jutarop Phetcharaburanin

Prof. Dr. Yupa Hanboonsong

Date: 8th July 2025

1. Introduction

Nowadays, the problem of antimicrobial resistance is increasing, caused by various factors related to the use of antibiotic drugs, including misuse, overuse, and long-term use of antibiotics to treat infectious diseases in humans. Moreover, antibiotics are used in agriculture and livestock to promote growth, treat, and prevent bacterial infections in animals (Ahmed et al., 2024; Okaiyeto et al., 2024; Xiong, Sun, & Zeng., 2018). The overuse of antimicrobials in food-producing animals has been accepted as a contributing factor to the occurrence of resistant bacteria, which is another important factor leading to resistant bacteria and antibiotics remaining in food products, affecting subsequent consumers in the food web including humans, animals, and the environment (Lekagul et al.,2023).

Therefore, the poultry industry, as one of the significant sectors of animal production, represents the largest supplier of animal protein in the world, which leads to excessive antibiotic use for treatment and prevention (Rawat et al., 2024). In addition, poultry meat is widely consumed in Thailand. In 2022, Thailand ranked fourth in global exports of processed chicken meat (Noenchat, Direksin, & Sornplang, 2023). Currently, Thailand is considering implementing the Voluntary Optimization of Antimicrobial Consumption (VOAC) to lower antimicrobial use while prioritizing antimicrobial resistance prevention and control in agriculture and livestock (Lekagul et al., 2024). Consequently, to help reduce the potential issue, antimicrobial peptides are an interesting alternative in livestock to reduce the use of antibiotic drugs in raising animals.

Antimicrobial peptides (AMPs) are small molecules of proteins, containing about 10 to 100 amino acids, and have hydrophobic and hydrophilic sides in molecules that show the character of amphipathic. AMPs play an important role in innate immunity and serve as the first line of defense against a wide spectrum of microorganisms. Furthermore, they exhibit strong antimicrobial activity against antibiotic-resistant bacteria, such as methicillin-resistant bacteria *Streptococcus aureus* and *Pseudomonas aeruginosa* (Manniello et al., 2021; Moretta et al., 2020; Park & Yoe, 2017; Silveira, Roque-Borda, & Vicente, 2021). Mostly, AMPs interact with bacterial membranes and penetrate lipid membranes in various ways, ultimately leading to cell death (Silveira, Roque-Borda, & Vicente, 2021). AMPs can be found in various organisms, including mammals, plants, amphibians, and insects (Nayab et al., 2022). Several insects possess notable antimicrobial properties and occur in higher proportions relative to other animal groups. Thus, the black soldier fly has been recognized as one of the primary sources of AMPs among insects (Pimchan et al., 2024).

Black Soldier Fly (*Hermetia illucens*; BSF) is found in tropical and warm-temperate regions and is considered a non-invasive insect (Elhag et al., 2017). BSF contains a large number of identified AMPs, such as Hidefensin-1, Hidiptericin-1, Cecropin 1, Hill-Cec1, and Hill-Cec10, which belong to the defensins and cecropin families (Fahmy et al., 2024; Pimchan et al., 2024). Mostly, the mode of action of defensins is active against Gram-positive bacteria, some are also active against Gram-negative bacteria and fungi, while cecropins are mainly active against Gram-negative bacteria, less effective against Gram-positive bacteria, and some cecropins have antifungal activity (Fahmy et al., 2024; Manniello et al., 2021). The mechanisms of AMPs are always related to disrupting membranes and interacting with intracellular compounds (Fahmy et al., 2024; Pimchan et al., 2024). Moreover, BSF has the capability to generate efficient AMPs while also serving as a valuable resource, being rich in proteins, essential amino acids, and fatty acids. Therefore, BSF larvae are used as a feed source for livestock, including pigs, fish, and poultry. (Muslykhah et al., 2024; Shah et al., 2023).

This study aims to immunize BSF larvae with bacteria for AMP production, characterize the AMPs, and determine their ability to inhibit bacterial pathogens. Previous research has demonstrated that stimulating AMP production in BSF larvae *via* hemolymph, by simultaneously challenging them with *Escherichia coli*, *Staphylococcus aureus*, and a combination of *E. coli* and *S. aureus*, significantly enhances their immunity (Ho et al., 2021).

Thus, this study focuses on enhancing the immunity of BSF larvae through both hemolymph injection and feeding under different conditions, including live bacteria, heat-killed bacteria, and different bacterial-nanoparticles. Then, the optimal condition and the ability to inhibit poultry-related pathogens will be selected. Following this, the characteristics of AMPs extracted from BSF larvae were analyzed, leading to the application of BSF larvae as a supplemental source in poultry feed. This approach aims to enhance chicken immunity and provide an alternative strategy to reducing antibiotic use in livestock and poultry farming.

2. Objectives

1. To evaluate antimicrobial peptide production in BSF larvae, compare between hemolymph and feed method

2. To determine the antibacterial activity of antimicrobial peptides against bacterial pathogens, compare between hemolymph and feeding immunization

3. To investigate the structure and functions of AMPs from BSF larvae

4. To assess the potential of BSF larvae as a supplementary source in chicken feed

3. Materials and methods

Materials

Black soldier fly (*Hermetia illucens*) larvae (BSF larvae) and chicken feed diet including Formula A; palm kernel meal: brewer's grain: cassava (60:20:20) and Formula B; palm kernel meal: brewer's grain (60:40) (Agriculture, Khon Kaen University, Thailand). Live cells (mixed bacteria; *S. aureus* and *E. coli* 1× 10^3 CFU/ml) and 1x phosphate buffer (PBS). Bacterial nanoparticles, including Formula 1 killed bacteria coated with chitosan (F1), Formula 2 killed bacteria encapsulated in cationic Lipid nanoparticles (F2), and Formula 3 killed bacteria encapsulated in cationic Lipid nanoparticles coated with fucoidan (F3) supported by the National Nanotechnology Center.

Methods

1. Immunization of BSF larvae

1.1 Immunization *via* the hemolymph

BSF 10-day-old larvae were cleaned with 70% alcohol 1 time for 30 min, distilled water (DW) 3 times, and dried. BSF larvae were injected with 10 µL of hemolymph (Ho et al., 2021) for three conditions, including F1, F3, and 1x phosphate buffer (PBS) used as a control

group (~600 BSF larvae/condition). Observation 30 min, BSF larvae were divided into two groups and reared in a tray filled with Formula A and Formula B feeds, at room temperature for 36 h (BSF larvae 125 g) and 72 h (BSF larvae 75 g). After incubation at the time point, BSF larvae were cleaned three times with sterile water and stored for lyophilization before subsequent extraction of crude AMPs.

1.2 Immunization with feedings

The three conditions described above (F1, F3, and 1xPBS), with a prepared volume of 40 ml, were added to 200 g of Formula A and Formula B feeds. BSF 10-day-old larvae 200 g (~1200 larvae) per condition were reared in a tray, incubated at room temperature for 72 h. End of the experiment, separate the BSF larvae from each feed, clean them with sterile water three times, and keep them at −20 °C. Then, the BSF larvae are lyophilized and stored for extraction and analysis. The enumeration of *E.coli* and *S.aureus* present in the feed was determined using Petrifilm™ Staph Express Count Plate and Petrifilm™ *E. coli*/Coliform Count Plate; Neogen.

2. Analysis of crude AMP extraction

2.1 BCA analysis for total protein quantification

From the BCA analysis (Bicinchoninic acid assay) for total protein quantification, the crude AMPs obtained under the conditions of 1xPBS, heat-killed cells, and live cells were dissolved in DI water to a final concentration of 500 mg/ml. The samples were then analyzed using the Pierce™ 660nm Protein Assay Kit (Thermo Scientific™)

2.2 Disc diffusion assay

AMPs solution was prepared by dissolving crude AMPs obtained from the 1xPBS, heat-killed cells, and live cells conditions in DI water to the following concentrations: 500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml. *S. aureus*, *E. coli*, and MRSA 1-1463 were cultured in MHB (Mueller Hinton broth) to mid-log phase, then adjust to 0.5 McFarland. The bacterial suspension was soaked by cotton swab, then swab the suspension onto an MHA plate (Mueller Hinton Agar; 25 ml), place a 6 mm paper disk on the plate, and drop 10 µL of each AMPs solution onto the paper disk. Ampicillin 10 µg and DI water were used for positive and negative control, respectively. The plates were incubated at 37 °C for 18 h and measured the inhibition zone.

4. Results

4.1 Wet weight and BSF larvae mortality

Based on the previous progression, the sample quantity was insufficient for antibacterial activity analysis. Therefore, the experiment was repeated, with the immunization of BSF larvae via hemolymph and feeding, following conditions: 1xPBS (control), F1, and F3.

From the experiment, wet weight gain was recorded for each condition after immunization. Firstly, in the 36 h post-injection group, the wet weight of the larvae decreased across all conditions, ranging from 6.5 to 17.3 g. (Table 1). Then, larvae fed with Feed A showed lower wet weight gain compared to those fed with Feed B, which is consistent with the higher number of dead larvae observed in this group. The injection and formula solution can induce the immune system, which may cause injuries, leading to larval mortality. In contrast, Feed B appears to stimulate metabolic activity in BSF larvae, which may contribute to better survival and growth (Ho et al., 2021). The result of wet weight and BSF larval mortality 36 h post-injection under each experimental condition is shown in Table 1.

Table 1: Wet weight and larval mortality 36 h post-injection under each experimental condition

Number	Conditions	Initial wet weight (g)	Post-wet weigh (g)	Weight gain (g)	Number of dead larvae (larva)
1	A-PBS	125.0	107.7	-17.3	21
2	A-F1	125.0	106.0	-19.1	18
3	A-F3	125.0	105.2	-19.8	27
4	B-PBS	127.4	125.7	-1.7	24
5	B-F1	127.6	119.4	-8.2	33
6	B-F3	129.9	123.4	-6.5	15

The results of the rate of wet weight change 72 h post-injection for each experimental condition (Fig. 1) show that larvae fed with Feed B exhibited higher wet weight gain, ranging from 23.32% to 30.24%, whereas those fed with Feed A showed a lower increase, 0.60 to 2.41 %. Consistently, under feeding conditions (Fig.2), larvae fed with Feed B displayed a higher rate of wet weight change 72 h after feeding, from 47.71 to 55.46%.

Therefore, immunization of BSF larvae *via* feeding methods can result in greater weight gain, indicating a higher survival rate. Additionally, observations of the mortality rate under different experimental conditions (Fig. 3) show that larvae immunized *via* the feeding method had the lowest mortality, followed by the 72 h post-injection and 36 h post-injection groups, respectively.

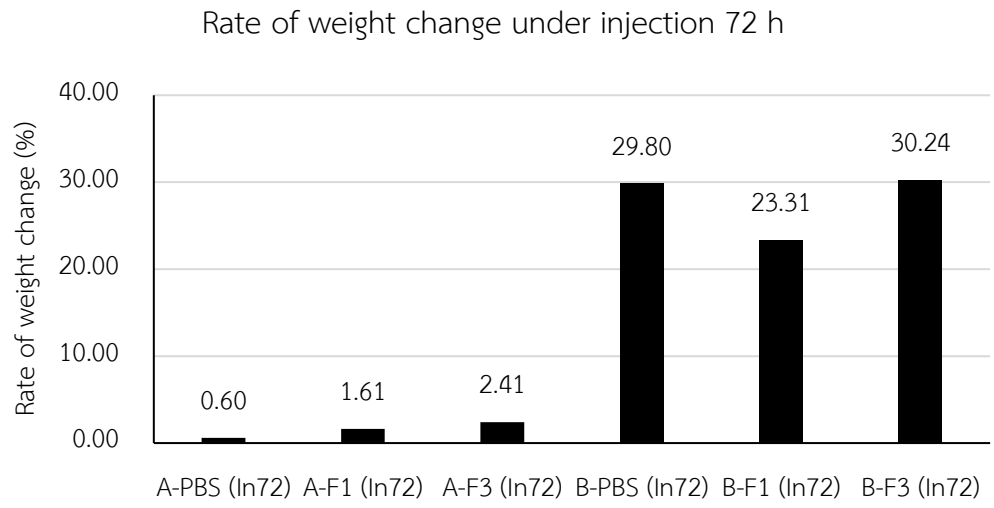


Fig. 1. The rate of wet weight change 72 h post-injection for each experimental condition

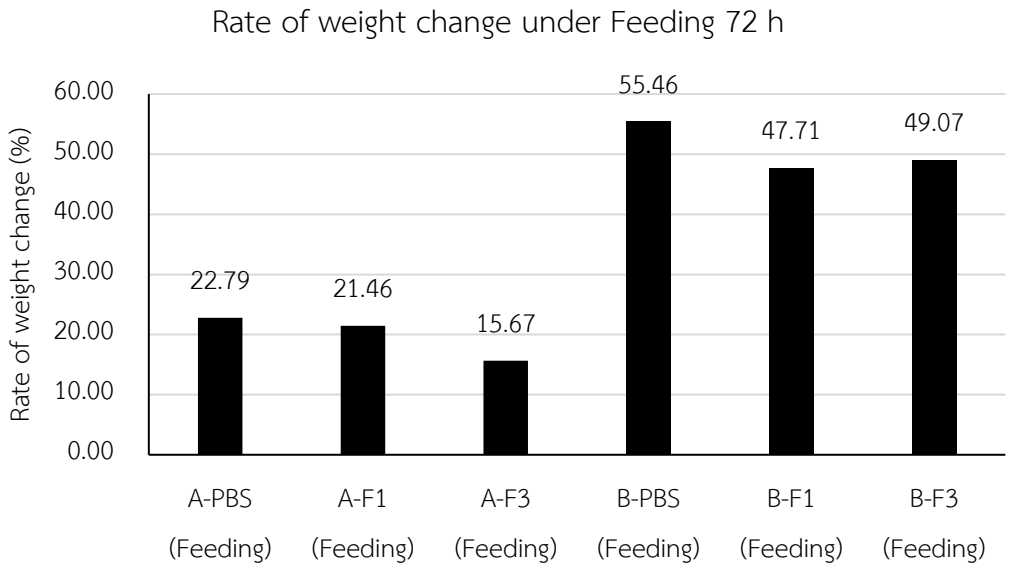


Fig. 2. The rate of wet weight change 72 h after feeding for each experimental condition.

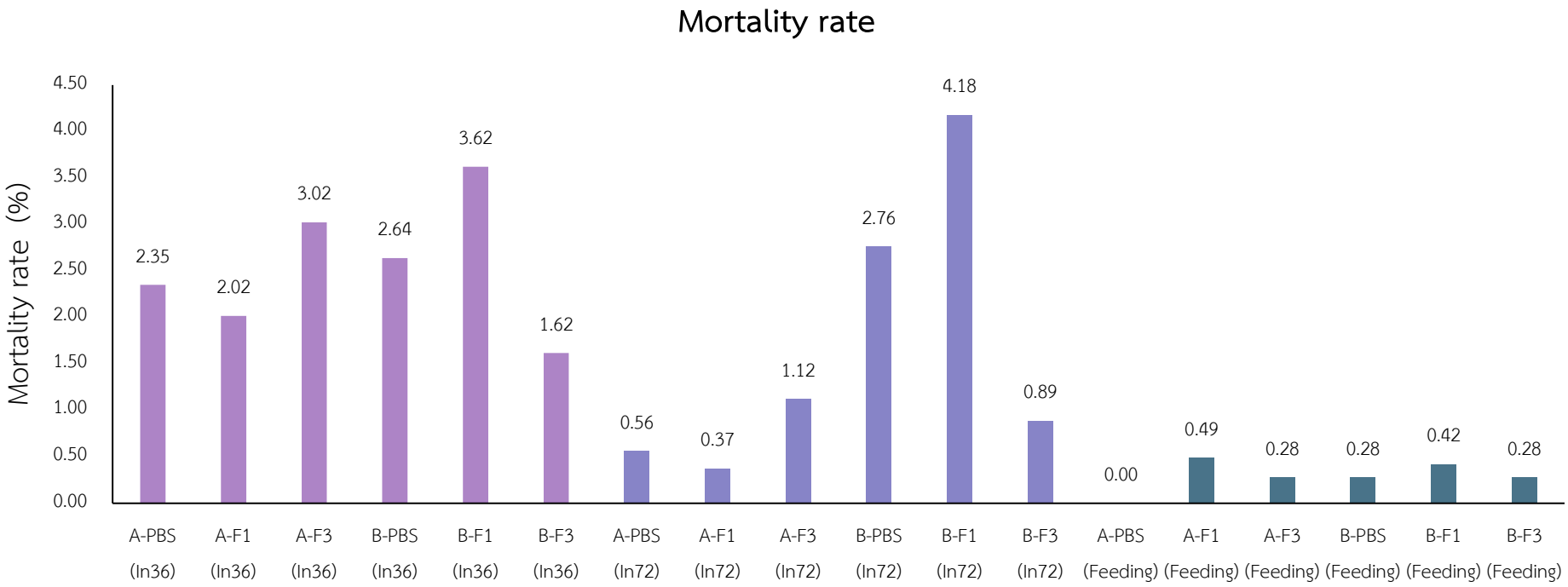


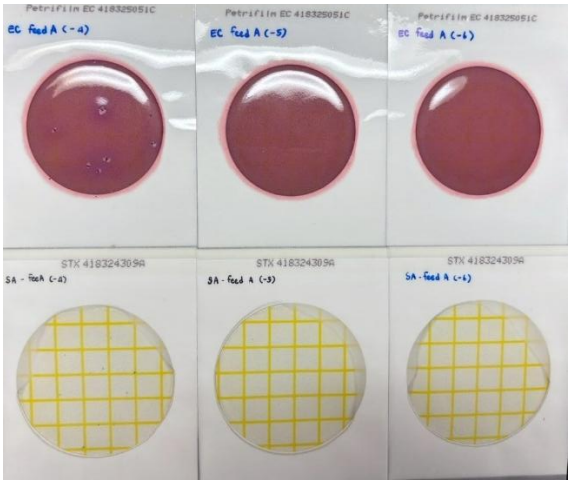
Fig. 3. The mortality rate under different experimental conditions.

(A= Feed A, B= Feed B, In36= Injection 36 h condition, and In72= Injection 72 h condition)

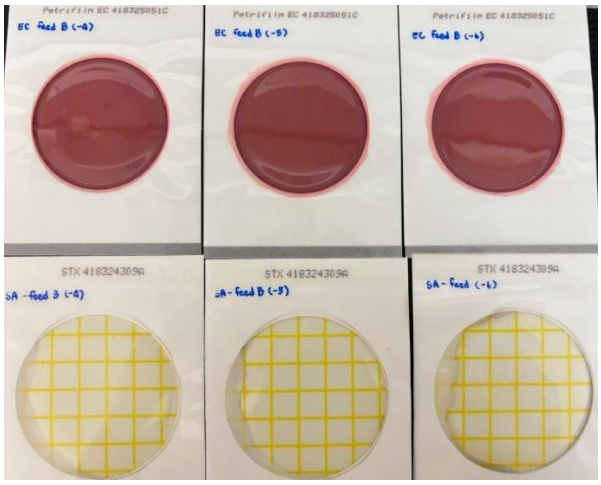
4.2 Bacterial culture from feeding

The presence of *S. aureus* and *E. coli* in the feed was investigated using Petrifilm™ Staph Express Count Plate and Petrifilm™ *E. coli*/Coliform Count Plate, respectively. These results may be related to the production of AMPs by BSF larvae. Figures 4A and 4B show that no *S. aureus* and *E. coli* colonies were detected on day 0 of the initial experiment in both Feed A and Feed B.

In contrast, on day 3 of the experiment, *S. aureus* and *E. coli* colonies were observed in both feeds (Fig. 5A and 5B). Quantitative analysis showed $6x10^4$ CFU/ml of *E.coli* in Feed A and 1.04 $x10^6$ CFU/ml of *S. aureus* and $1.5x10^5$ CFU/ml of *E.coli* in Feed B.

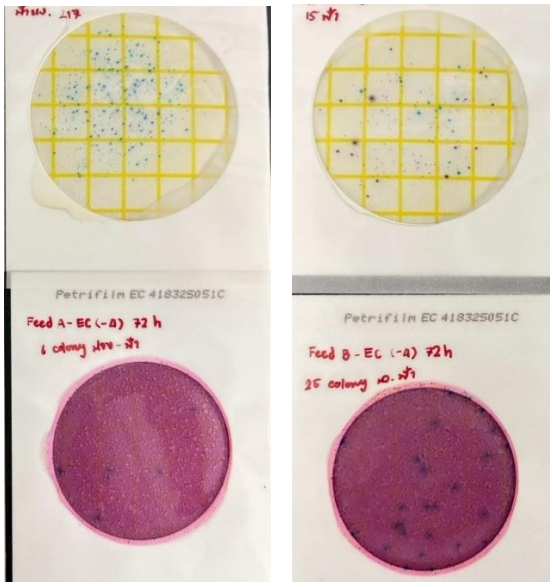


(A.)



(B.)

Fig. 4. Results of Petrifilm™ Staph Express and *E.coli*/Coliform Count Plate on day 0 of experiment, (A; Feed A, and B; Feed B)



(A.) (B.)

Fig. 5. Results of Petrifilm™ Staph Express and *E.coli*/Coliform Count Plate on day 3 of experiment, (A; Feed A, and B; Feed B)

4.3 The analysis of crude AMP extraction

4.3.1 BCA analysis for total protein quantification

Protein quantification was performed on crude AMPs obtained from 18 different experimental conditions, including injection at 36 h and 72 h post-injection, as well as feeding methods. Each method was further divided into PBS (control), heat-killed cells, and live cells. The results were analyzed by comparing the measured protein concentrations with the Bovine serum albumin (BSA) standard curve.

Protein concentrations of crude AMP was measured under different conditions and the results as shown in Figures 6, 7, and 8. Under the injection 36 h condition, the protein concentrations ranged from 269.60 to 496.27 µg/ml (Fig.6). No significant differences in protein concentration were observed between feed A and feed B (p-value = 0.078). In contrast, under the injection 72 h, a significant difference was observed between the groups (p-value <0.01). Feed B exhibited higher protein concentration, ranging from 521.80 to 739.33 µg/ml. However, within feed B, the heat-killed cells condition did not different significance from the live cells condition (Fig.7). For the feeding method, the protein concentration ranged from 91.00 to 658.67 µg/ml (Fig.8). Feed A under the PBS condition showed the highest protein concentration. Following, Feed B under the PBS and heat-killed cells condition, as well as Feed A under the live cells condition, shows no significant differences

from the other group. However, the lowest protein concentrations were observed in the Feed B live cells and the Feed A heat-killed cells.

Moreover, protein content (%) was calculated from 500 mg/ml crude AMP extracts (Table 2), ranging from 0.02% to 0.15%. these results suggest that the crude AMP extracts may contain a high proportion of other components, such as cell fragments, cell debris, water, and residual solutions.

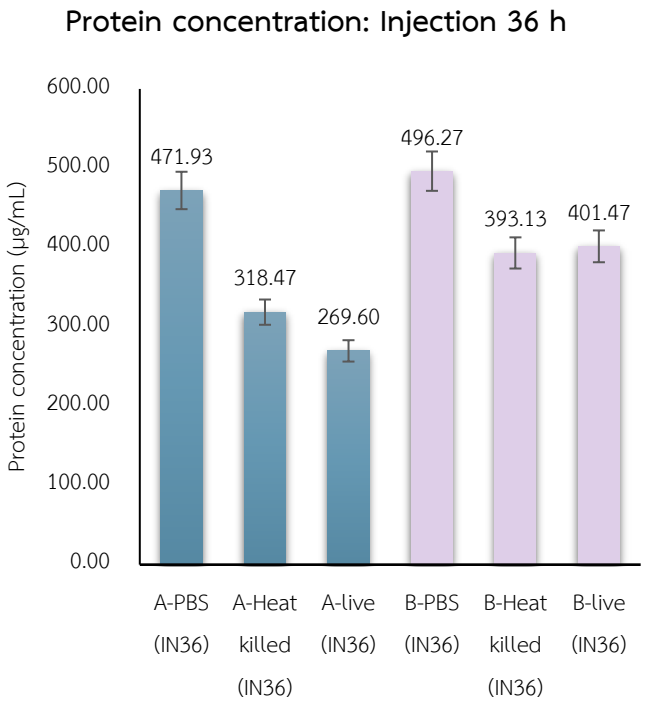


Fig. 6 Protein concentration for the injection 36 h method measured by BCA assay under different experimental conditions; p-value = 0.078 (A= Feed A, B= Feed B)
(One-way ANOVA, p value <0.05); N=3

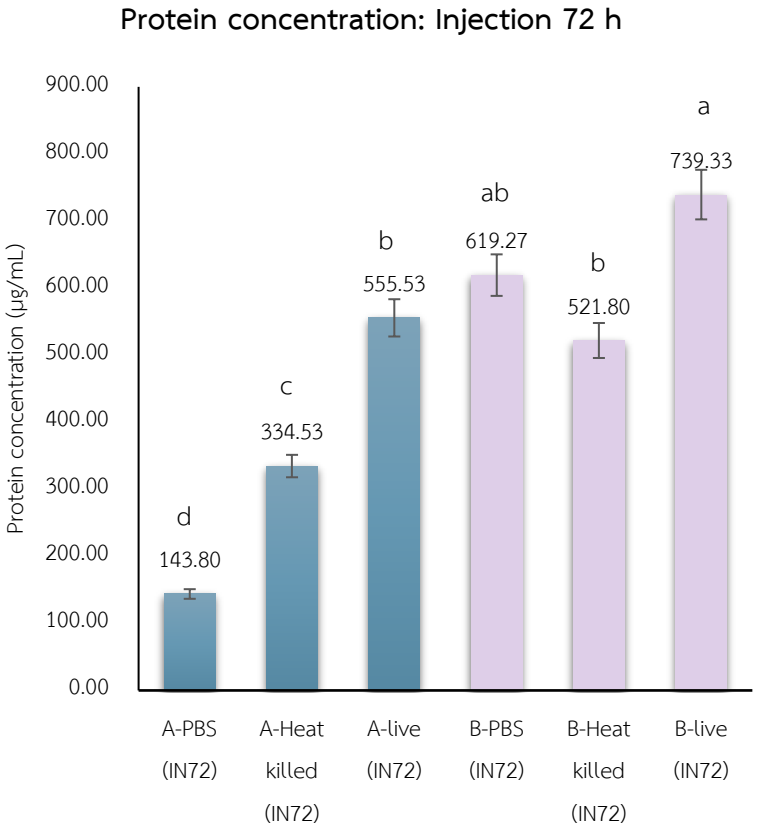


Fig. 7 Protein concentration for the injection 72 h method measured by BCA assay under different experimental conditions; p-value <0.01 (A= Feed A, B= Feed B)

*One-way ANOVA, p-value <0.05); N=3

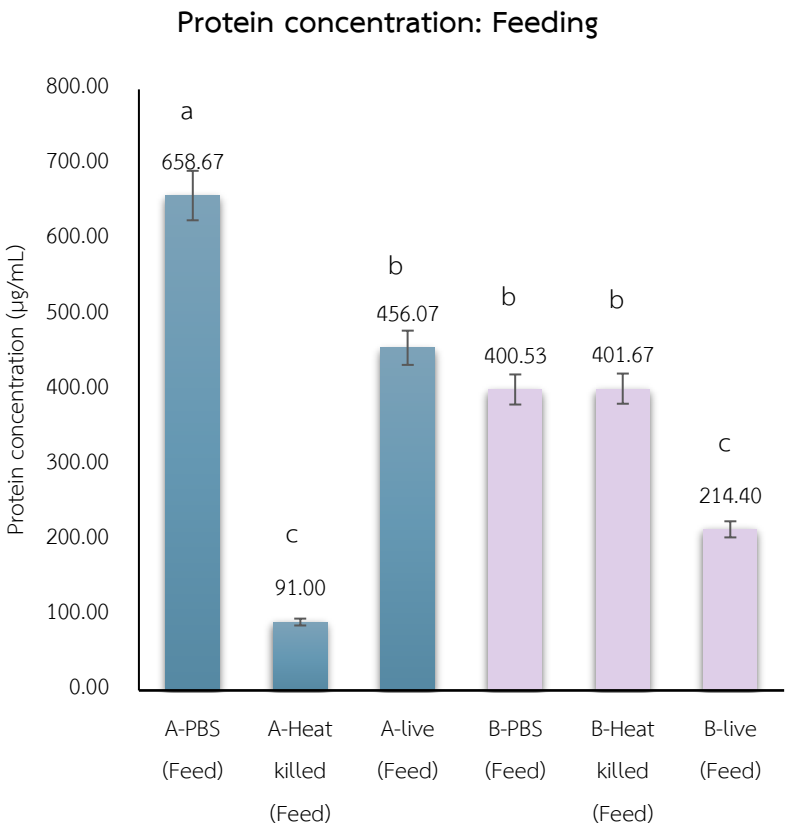


Fig. 8 Protein concentration for the Feeding method measured by BCA assay under different experimental conditions; p-value <0.01 (A= Feed A, B= Feed B)

*One-way ANOVA, p-value <0.05); N=3

Table 2: Protein content (%) in 500 mg/ml Crude AMPs under different experimental conditions

Methods	Conditions	Preparation from crude proteins (mg/ml)	Protein concentration (µg/mL)	Protein content (%)
Injection 36 h.	A-PBS	500	471.93	0.09
	A-Heat killed	500	318.47	0.06
	A-live	500	269.60	0.05
	B-PBS	500	496.27	0.10
	B-Heat killed	500	393.13	0.08
	B-live	500	401.47	0.08
Injection 72 h.	A-PBS	500	143.80	0.03
	A-Heat killed	500	334.53	0.07
	A-live	500	555.53	0.11
	B-PBS	500	619.27	0.12
	B-Heat killed	500	521.80	0.10
	B-live	500	739.33	0.15
Feeding	A-PBS	500	658.67	0.13
	A-Heat killed	500	91.00	0.02
	A-live	500	456.07	0.09
	B-PBS	500	400.53	0.08
	B-Heat killed	500	401.67	0.08
	B-live	500	214.40	0.04

*A= Feed A, B= Feed B

4.3.2 Disc diffusion assay

AMP solutions were prepared by dissolving crude AMPs obtained from the 1xPBS, heat-killed cells, and live cells conditions in DI water to the following concentrations: 500 mg/ml, 250 mg/ml, 125 mg/ml, and 62.5 mg/ml, ampicillin (10 μg) was used as the positive control, and DI water served as the negative control. The antibacterial activity of these samples was evaluated against *S. aureus*, *E. coli*, and MRSA 1-1463.

Table 3 presents the disc diffusion results, showing that no inhibition zone was observed against *E. coli* under any condition or method. Furthermore, inhibition against MRSA 1-1463 was detected only in the Feed B heat-killed cell (36 h injection method) at a concentration of 500 mg/ml, producing an inhibition zone of 7.00 mm. Thus, the disc diffusion assay against *S. aureus.* DI water, used as the negative control, exhibited no inhibition zone (6.00 mm), whereas ampicillin (10 μg), as the positive control, produced inhibition zones ranging from 24.67 to 15.00 mm. Among the crude AMPs at 500 mg/ml, all experimental conditions showed inhibitory activity except for the Feed A heat-killed cells (Feeding method), which may result from processing dissolved crude AMP, and the lowest protein concentration observed in this sample, consistent with the BCA protein quantification results. The 500 mg/ml crude AMP solutions produced a diffuse inhibition zone against *S. aureus*, ranging from 10.67 to 7.67 mm. Thus, the largest inhibition zone (10.67 mm) was observed in the Feed B PBS and heat-killed cells under the 36h injection method. These findings suggest that the injection method, which directly delivers immunized AMPs into the hemolymph, may enhance AMP production and result in stronger antimicrobial activity.

Table 3: Disc diffusion assay against *S. aureus*, MRSA1-1463, and *E.coli* using 500 mg/ml crude AMPs under different experimental conditions

Methods	Number	Conditions	500 mg/ml crude AMPs inhibition zone (mm)		
			S. aureus	MRSA1-1463	*E. coli*
Injection 36 h.	1	A-PBS	10.67	6.00	6.00
	2	A-Heat killed	10.00	6.00	6.00
	3	A-live	7.67	6.00	6.00
	4	B-PBS	10.67	6.00	6.00
	5	B-Heat killed	10.67	7.00	6.00
	6	B-live	10.33	6.00	6.00
Injection 72 h.	7	A-PBS	8.67	6.00	6.00
	8	A-Heat killed	8.67	6.00	6.00
	9	A-live	9.33	6.00	6.00
	10	B-PBS	8.33	6.00	6.00
	11	B-Heat killed	8.67	6.00	6.00
	12	B-live	9.33	6.00	6.00
Feeding	13	A-PBS	8.67	6.00	6.00
	14	A-Heat killed	6.00	6.00	6.00
	15	A-live	9.00	6.00	6.00
	16	B-PBS	10.00	6.00	6.00
	17	B-Heat killed	8.67	6.00	6.00
	18	B-live	8.67	6.00	6.00

*Disc size = 6.00 mm

*DI water, used as the negative control, no inhibition zone (6.00 mm)

*Ampicillin (10 μg), as the positive control

5. Conclusions

From observation of immunization of BSF larvae, the 36 h post-injection group exhibited reduced wet weight and a high mortality rate. Due to the injection and formula solution inducing an immune response that may cause injury, ultimately leading to larval death. In contrast, larvae in the 72 h injection group and the Feeding methods showed an increase in wet weight gain and a lower mortality rate. These findings indicate that longer harvesting periods and feeding methods are more suitable for adaptation and larval survival.

Furthermore, analysis of the crude AMP extracts using the BCA analysis showed that the total protein content ranged from 0.02% to 0.15%, indicating that the crude AMP extracts contained a high proportion of non-protein components. In addition, the disc diffusion assay demonstrated that the 500 mg/ml crude AMP solutions produced a diffuse inhibition zone against *S. aureus*, ranging from 10.67 to 7.67 mm. The largest inhibition zone (10.67 mm) was observed in the Feed B PBS and heat-killed cells under the 36h injection method. These findings indicate that the crude AMP extracts possess inhibitory activity against *S. aureus.* Furthermore, Feed B heat-killed cell (36 h injection method) at a concentration of 500 mg/ml, inhibition against MRSA 1-1463 the producing an inhibition zone of 7.00 mm. However, the results of the disc diffusion assay indicated that further repetitions are needed to obtain clearer and more reliable antimicrobial activity data.

Further plan: BSF larvae will be immunized with F2 using all methods, including injection at 36 h and 72 h, and feeding. Then extraction crude AMPs, Crude AMPs will be extracted again, and the samples will be used for analyses, including amino acid composition using LC MS/MS. The antibacterial activity will also be evaluated again, including disc diffusion assay, MIC, and MBC.

6. References

Ahmed, S. K., Hussein, S., Qurbani, K., Ibrahim, R. H., Fareeq, A., Mahmood, K. A., & Mohamed, M. G. (2024). Antimicrobial resistance: Impacts, challenges, and future prospects. **Journal of Medicine, Surgery, and Public Health**, 2, 100081.

Elhag, O., Zhou, D., Song, Q., Soomro, A. A., Cai, M., Zheng, L., & others. (2017). Screening, expression, purification, and functional characterization of novel antimicrobial peptide genes from Hermetia illucens (L.). **PLoS ONE**, 12(1), e016958. https://doi.org/10.1371/journal.pone.016958

Fahmy, L., Generalovic, T., Ali, Y. M., Seilly, D., Sivanesan, K., Kalmar, L., Pipan, M., Christie, G., & Grant, A. J. (2024). A novel family of defensin-like peptides from Hermetia illucens with antibacterial properties. **BMC Microbiology**, 24(1), 167. https://doi.org/10.1186/s12866-024-03325-1

Ho, P. N., Klanrit, P., Hanboonsong, Y., Yordpratum, U., Suksawat, M., Kulthawatsiri, T., Jirahiranpat, A., Deewai, S., Mackawan, P., Sermswan, R. W., Namwat, N., Loilome, W., Khampitak, T., Wangwiwatsin, A., & Phetcharaburanin, J. (2021). Bacterial challenge associated metabolic phenotypes in Hermetia illucens defining nutritional and functional benefits. **Scientific Reports**, 11(1), 23316. https://doi.org/10.1038/s41598-021-02752-8

Lekagul, A., Kirivan, S., Kaewkhankhaeng, W., Khotchalai, S., Mader, R., & Tangcharoensathien, V. (2024). Voluntary optimisation of antimicrobial consumption in swine and poultry production in Thailand: A policy analysis. **Frontiers in Veterinary Science**, 11, 1375127. https://doi.org/10.3389/fvets.2024.1375127

Lekagul A, Kirivan S, Tansakul N, Krisanaphan C, Srinha J, Laoprasert T, et al. (2023). Antimicrobial consumption in food-producing animals in Thailand between 2017 and 2019: The analysis of national importation and production data. **PLoS ONE** 18(4): e0283819. https://doi.org/ 10.1371/journal.pone.0283819

Manniello, M. D., Moretta, A., Salvia, R., Scieuzo, C., Lucchetti, D., Vogel, H., Sgambato, A., & Falabella, P. (2021). Insect antimicrobial peptides: Potential weapons to counteract the antibiotic resistance. **Cellular and Molecular Life Sciences**, 78(10), 4259–4282. https://doi.org/10.1007/s00018-021-03784-z

Moretta, A., Salvia, R., Scieuzo, C., Somma, A.D., Vogel, H., Pucci, P., et al. (2020). A bioinformatic study of antimicrobial peptides identified in the Black Soldier Fly (BSF) Hermetia illucens (Diptera:Stratiomyidae). **Scientific Reports**, 10, 16875.

Muslykhah, U., Phupaboon, S., Suriyapha, C., Matra, M., & Wanapat, M. (2024). Encapsulation of protein-based bioactive from black soldier fly for ruminant feeding. **Journal of Agriculture and Food Research**, 18, 101325. https://doi.org/10.1016/j.jafr.2024.101325

Nayab, S., Aslam, M. A., Rahman, S. U., Sindhu, Z. U. D., Sajid, S., Zafar, N., Razaq, M., Kanwar, R., & Amanullah. (2022). A review of antimicrobial peptides: Its function, mode of action, and therapeutic potential. **International Journal of Peptide Research and Therapeutics**, *28*(46). https://doi.org/10.1007/s10989-021-10325-6

Noenchat, P., Direksin, K., and Sornplang, P. (2023). The phenotypic and genotypic antimicrobial resistance patterns of Salmonella isolated from chickens and meat at poultry slaughterhouses in Japan and Thailand, **Veterinary World**, 16(7): 1527–1533.

Okaiyeto, S.A., Sutar, P.P., Chen, C., Ni, J.B., Wang, J., Mujumdar, A.S., et al. (2024). Antibiotic resistant bacteria in food systems: Current status, resistance mechanisms, and mitigation strategies. **Agriculture Communications 2**, 100027.

Park, S.I., & Yoe, S.M. (2017). A novel cecropin-like peptide from black soldier fly, *Hermetia illucens*: Isolation, structural and functional characterization. **Entomological Research**, 47, 115–124.

Pimchan, T., Hamzeh, A., Siringan, P., Thumanu, K., Hanboonsong, Y., & Yongsawatdigul, J. (2024). Antibacterial peptides from black soldier fly (*Hermetia illucens*) larvae: Mode of action and characterization. **Scientific Reports**, 14(1), 12345. https://www.nature.com/articles/s41598-024-03456-w

Rawat, N., Anjali, A., Shreyata, S., Sabu, B., Bandyopadhyay, A., & Rajagopal, R. (2024). Assessment of antibiotic resistance in chicken meat labelled as antibiotic-free: A focus on *Escherichia coli* and horizontally transmissible antibiotic resistance genes. **LWT - Food Science and Technology**, 194, 115751. https://doi.org/10.1016/j.lwt.2024.115751

Shah, P. N., Ruan, X., van Loon, J. J. A., & Dicke, M. (2023). Temperature-modulated host-pathogen interactions between *Hermetia illucens* L. (Diptera: *Stratiomyidae*) and *Pseudomonas protegens* Pf-5. **Journal of Invertebrate Pathology**, 198, 107934. https://doi.org/10.1016/j.jip.2023.107934

Silveira, R. F., Roque-Borda, C. A., & Vicente, E. F. (2021). Antimicrobial peptides as a feed additive alternative to animal production, food safety, and public health implications: An overview. **Animal Nutrition**, 7(3), 896–904. https://doi.org/10.1016/j.aninu.2021.06.009

Xiong, W., Sun, Y., & Zeng, Z. (2018). Antimicrobial use and antimicrobial resistance in food animals. **Environmental Science and Pollution Research**, 25, 18377–18384.