Thesis Progression

Date: Dec17, 2025 Time: 11:00-11:30 AM

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

Presented by
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Introduction

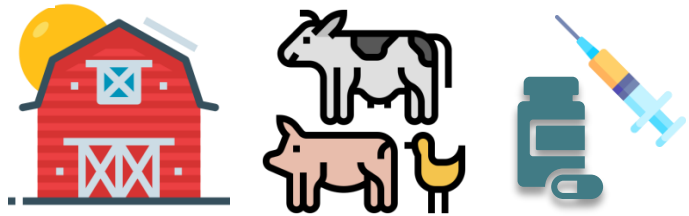
World Health Organization

Antimicrobial resistance 1.27 million global deaths in 2019

Cause by
Misuse, overuse, and long-term use of antibiotics in humans, **animals**, and plants.

Antibiotics used in agriculture and livestock

Concern



- Used to treat illness -Treat when symptoms appear
- Prophylactic use – to mitigate the risk of diseases that can result in animal illness or mortality

https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

Introduction

In livestock

The poultry farming

One of the significant sectors of animal production

The largest supplier of animal protein in the world

food production

Residue from animal excretion

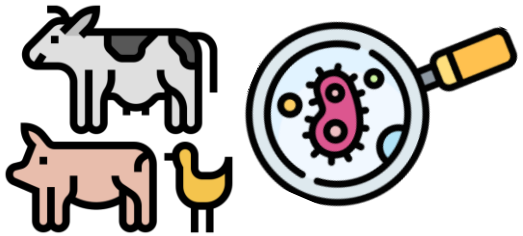
In 2022, Thailand ranked fourth in global exports of chicken meat

In 2016, the total amount of antibiotics used was estimated to be 161 tons.

(Wongsuvan et al., 2017)

(Noenchat, Direksin, & Sornplang, 2023)

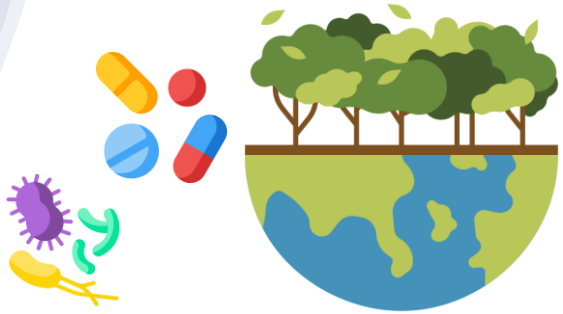
Problem: Antimicrobial resistance



- Affecting animal health
- May facilitate the emergence of antibiotic-resistant bacteria

affecting the next consumers, humans

- Received residual antibiotic
- May cause illness and death



Residue in the environment

Introduction

In livestock

The poultry farming

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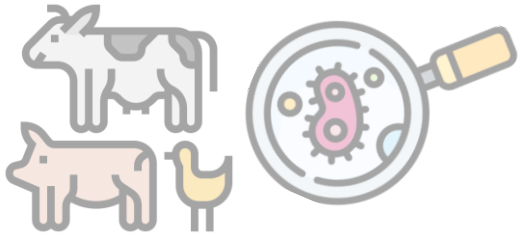
Problem: Antimicrobial resistance



- Affecting animal health
- May facilitate the emergence of antibiotic-resistant bacteria
- May cause illness and death

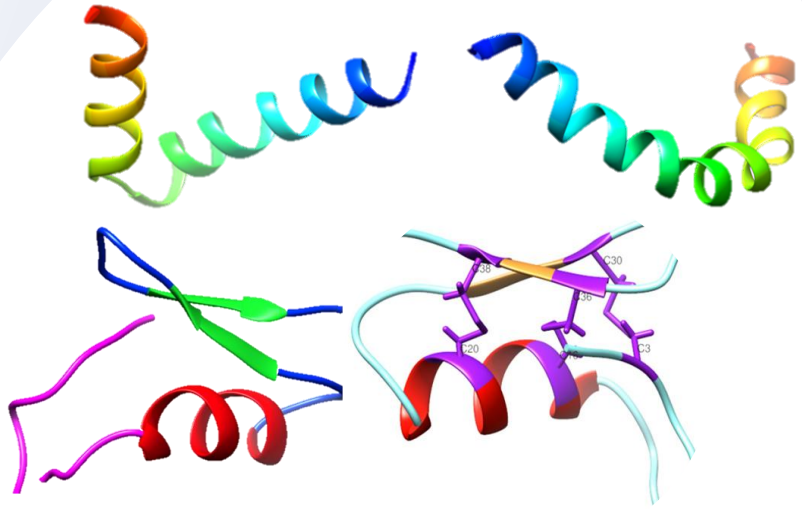
Residue in the environment

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 peptide

(AMPs)

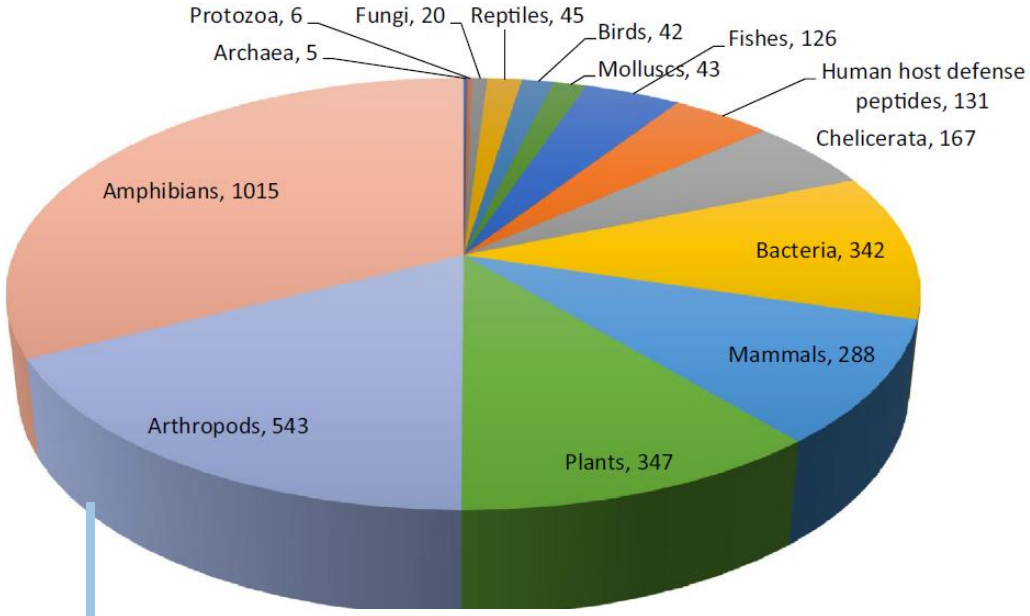
Small molecules: 10–100 amino acid residues

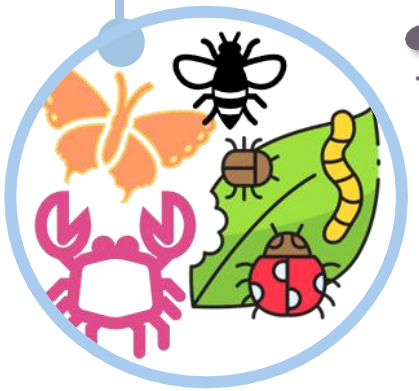


Structure and information of AMP: Manniello et al., (2021)

- Mostly, cationic (positive charge), hydrophobic, and hydrophilic
- Permeative components of the innate immune system
- Rapid action and show activity against bacteria, viruses, and fungi
- A diverse array of organisms, including amphibians, **insects**, plants, microorganisms, and mammals

Diversity of AMPs found in various organisms





Insects are one of the most famous sources of AMP

324 insect-derived AMPs

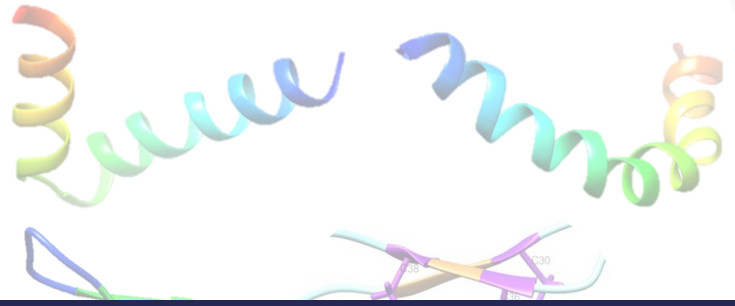
http:// aps. unmc. edu/ AP. 2019

Nayab et al., 2022 and Pimchan et al., 2024

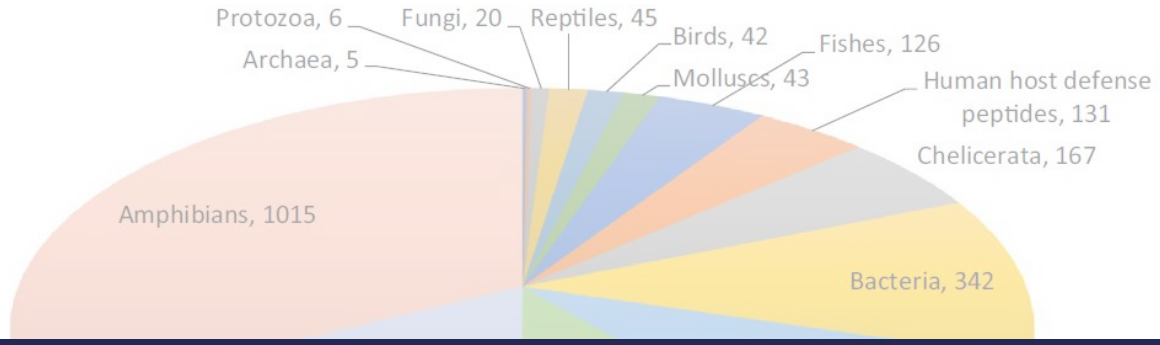
Antimicrobial peptide

(AMPs)

Small molecules: 10–100 amino acid residues



Diversity of AMPs found in various organisms



The proportion of AMPs in insects is high,
One of the most famous sources of AMPs among insects
Black soldier fly (BSF)

- Mostly, cationic (positive charge), hydrophobic, and hydrophilic
- Permeative components of the innate immune system
- Rapid action and show activity against bacteria, viruses, and fungi
- A diverse array of organisms, including amphibians, **insects**, plants, microorganisms, and mammals

Insects are one of the most famous sources of AMP
324 insect-derived AMPs

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Black soldier fly, BSF (*Hermetia illucens*)

BSF larvae

AMPs

Defensins: Defensin-like peptides (DLP1-4), Hidefensin-1, Hill-BB (C6571, C16634, C46948 and C7985)

Cecropins: Cecropin 1, Cecropin-like peptides (CLP1-3)

Attacins: HI-attacin

Sarcotoxin: Sarcotoxin 1, 2a, 2b and 3

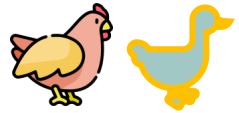
(Pimchan et al., 2024)

high nutrient values

- high protein content
- fat
- chitin
- vitamins
- minerals

Applications

The larvae can be used as animal feeds

- poultry 
- pigs
- fish 

Literature Reviews

AMPs were extracted from BSF larvae

www.nature.com/scientificreports

scientific reports

Check for updates

OPEN

Antibacterial peptides from black soldier fly (*Hermetia illucens*) larvae: mode of action and characterization

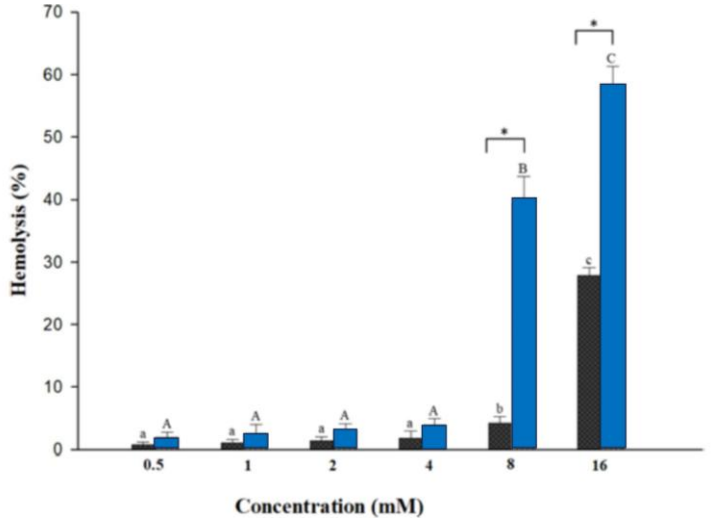
Thippawan Pimchan[1], Ali Hamzeh[1], Patcharin Siringan[1], Kanjana Thumanu[2], Yupa Hanboonsong[3] & Jirawat Yongsawatdigul[1]

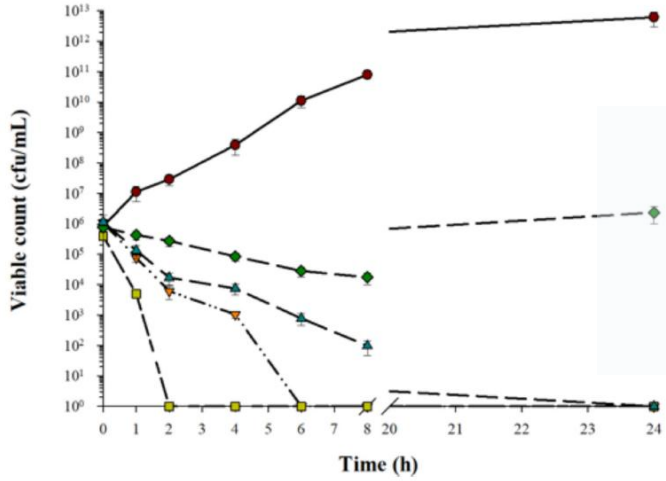
AMP showed antibacterial activity against

L. monocytogenes DMST 17303
S. Enteritidis DMST 15679
E. coli O157:H7 DMST 12743

Hemolytic activity in humans red blood cells

a hemolysis rate of less than 5%



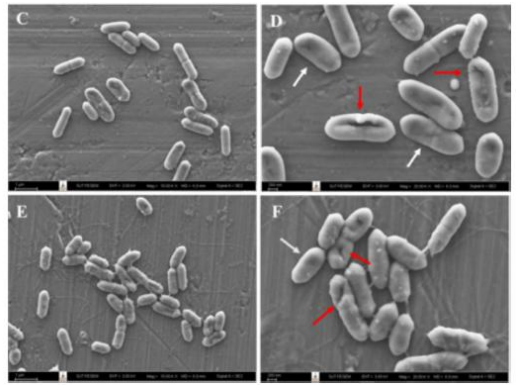


Time-killing kinetics against *L. monocytogenes*

within 2 h

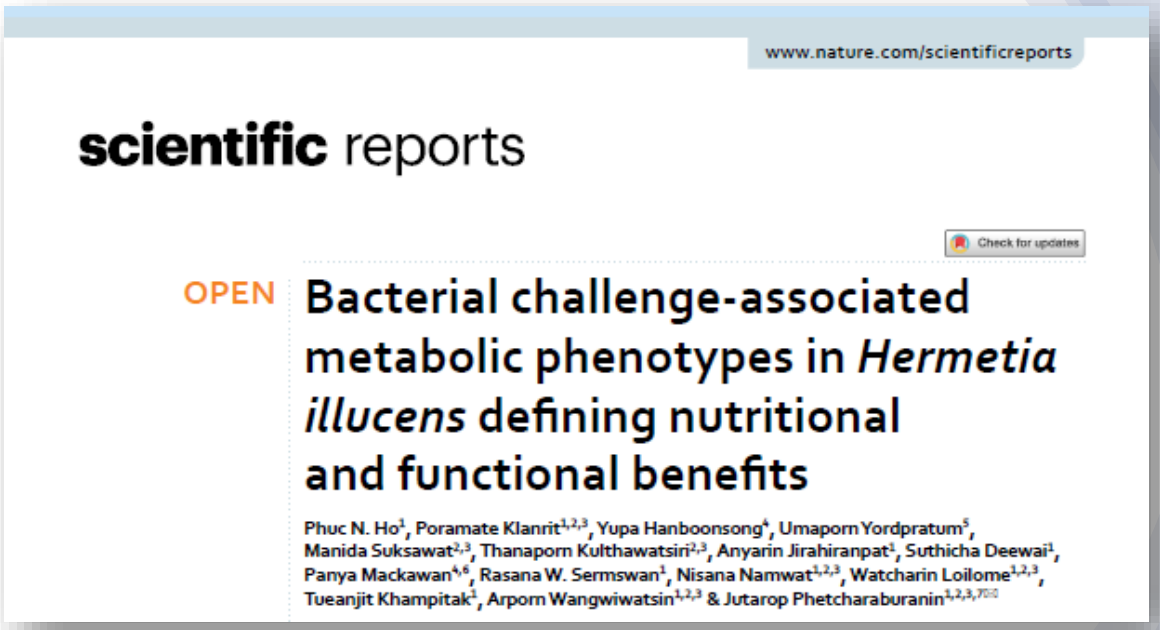
L. monocytogenes is the most susceptible

cell membrane disruption



Literature Reviews

BSF larvae in response to challenges


www.nature.com/scientificreports

scientific reports

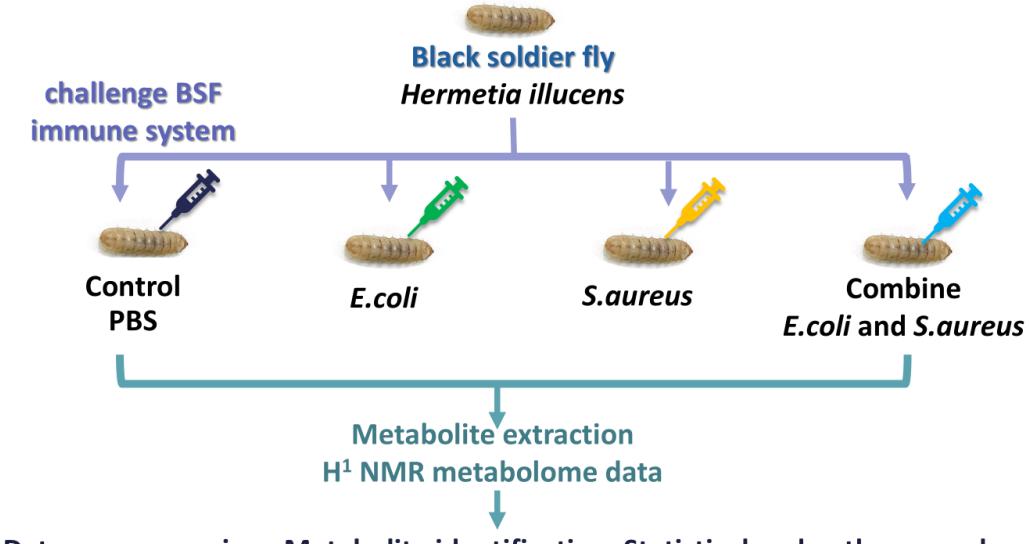
Check for updates

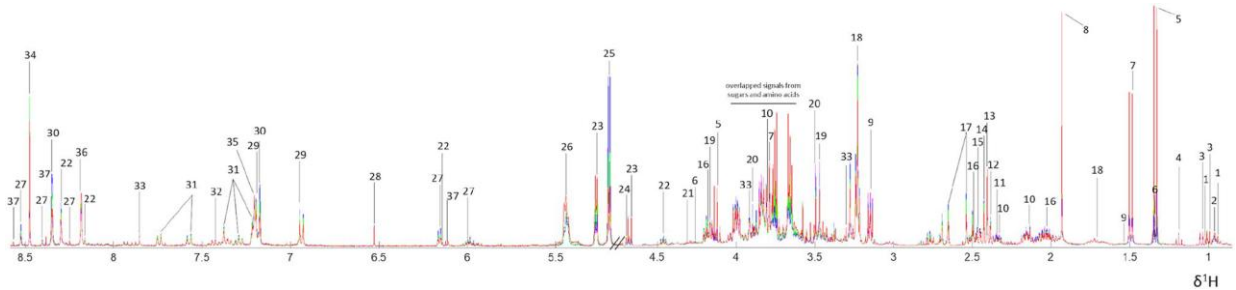
OPEN

Bacterial challenge-associated metabolic phenotypes in *Hermetia illucens* defining nutritional and functional benefits

Phuc N. Ho[1], Poramate Klanrit[1,2,3], Yupa Hanboonsong[4], Umaporn Yordpratum[5], Manida Suksawat[2,3], Thanaporn Kulthawatsiri[2,3], Anyarin Jirahiranpat[1], Suthicha Deewai[1], Panya Mackawan[4,6], Rasana W. Sermswan[1], Nisana Namwat[1,2,3], Watcharin Loilome[1,2,3], Tueanjit Khampitak[1], Arporn Wangwiwatsin[1,2,3] & Jutarop Phetcharaburanin[1,2,3,7]

***Escherichia coli*, *Staphylococcus aureus*, or a combined bacterial challenge**



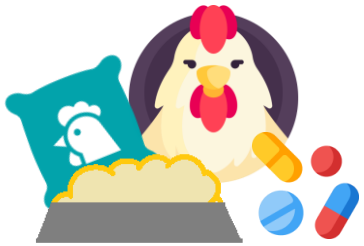


A total of 37 metabolites

characterized in the larvae, with key groups including amino acids, organic acids, and sugars

Conceptual framework

Problem



antimicrobial resistance associated with food production (the chicken feed)

GAP

Overuse of antibiotics in raising animals and livestock farming

antibiotics remain in foods and affect the next consumers or humans

Process

BSF larvae

Promote AMP production in BSF larvae

Stimulate the immune system with bacteria

\+

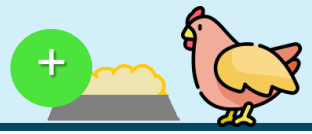
BSF larvae with effective AMP

Extraction

ability of AMPs to inhibit pathogens

Structural and functional analysis

applied as chicken feed



Output

- BSF larvae effectively produce AMPs by stimulating an immune response using bacterial nanoparticles.
- AMPs can inhibit pathogens associated with chicken infections, including *S. aureus, E. coli,* and MRSA.
- BSF larvae were used in supplemental chicken feed

Outcome

BSF larvae produce highly effective AMPs, which can be used as a chicken feed supplement to support and enhance chicken immunity.

Impact

BSF larvae can be used as a supplemental component in chicken feed, enhancing chicken immunity and potentially reducing antibiotic use in chicken farming, thereby contributing to reducing antimicrobial resistance in food production.

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 pathogen 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

The experimental design

Immunization of BSF larvae

Hemolymph vs **Feeding**

Hemolymph	Feeding
Live cells	Live cells
Heat kill	Heat kill
F1	F1
F2	F2
F3	F3
Control	Control

Part I

AMP extraction from immunized larvae

↓

Determined the amount of AMP from **Hemolymph vs Feeding**

Part II

Antibacterial activity of AMP from immunized larvae against chicken pathogen

↓

Disc diffusion
MIC
MBC

Part III

Characterization of AMP from immunized larvae

↓

Column separation

↓

Mass spectra photometry

Part IV

Application of immunized BSF larvae as supplement in chicken feed

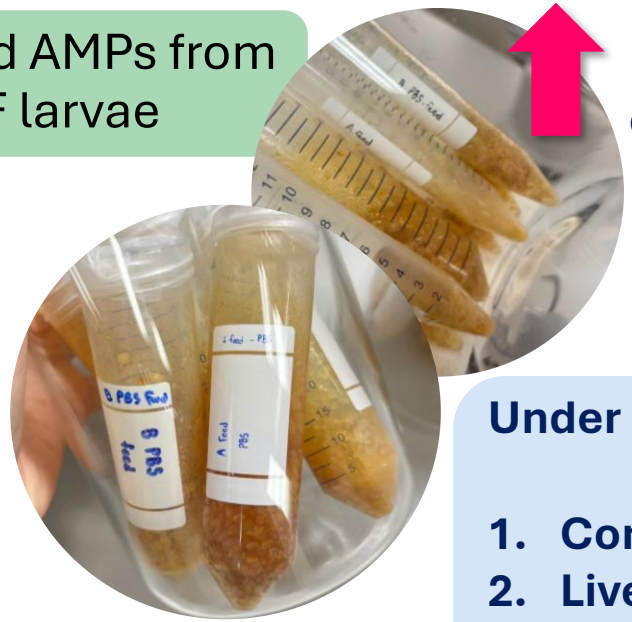
Bacterial nanoparticles

F1: Formula 1 killed bacteria coated with chitosan
F2: Formula 2 killed bacteria encapsulated in cationic Lipid nanoparticles
F3: Formula 3 killed bacteria encapsulated in cationic Lipid nanoparticles coated with fucoidan

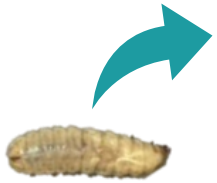
Previous progression

1

Extracted AMPs from BSF larvae



Increase crude AMPs



1. Immunization *via* hemolymph

2. Immunization *via* feeding

Under conditions:

1. **Control (PBS buffer)**
2. **Live cells**
3. **Heat kill**

2

crude AMPs

divide

Antibacterial activity test

(Part II)

- BCA analysis
- Disc diffusion assay

The samples are currently being submitted

for LC-MS/MS analysis

Immunized again:

Immunization *via* hemolymph and feeding

Under conditions: F1, F2, and F3 (from Nanotech)

Extracted AMPs

Increase the amount of crude AMPs extract collected

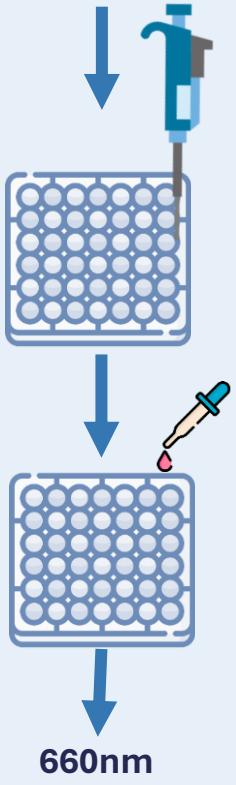
Methods: Analysis of crude AMP extraction

1. BCA analysis for total protein quantification

crude AMPs

dissolving in DI water

Final concentrations
500 mg/ml



The Pierce™ 660nm Protein Assay Kit (Thermo Scientific™)

660nm

2. Disc diffusion assay

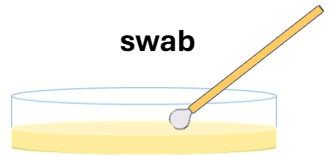
Crude AMPs include 18 conditions

	Number	Conditions
Injection 36 h.	1	A-PBS
	2	A-Heat killed
	3	A-live
	4	B-PBS
	5	B-Heat killed
	6	B-live
Injection 72 h.	Number	Conditions
	7	A-PBS
	8	A-Heat killed
	9	A-live
	10	B-PBS
	11	B-Heat killed
	12	B-live
Feeding	Number	Conditions
	13	A-PBS
	14	A-Heat killed
	15	A-live
	16	B-PBS
	17	B-Heat killed
	18	B-live

dissolving in DI water

Final concentrations:
500 mg/ml
250 mg/ml
125 mg/ml
62.5 mg/ml

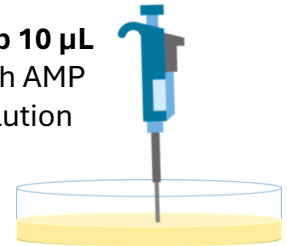
swab



0.5 McFarland
S. aureus
E. Coli
MRSA 1-1463

Mueller Hinton Agar 25 ml

Drop 10 µL each AMP solution

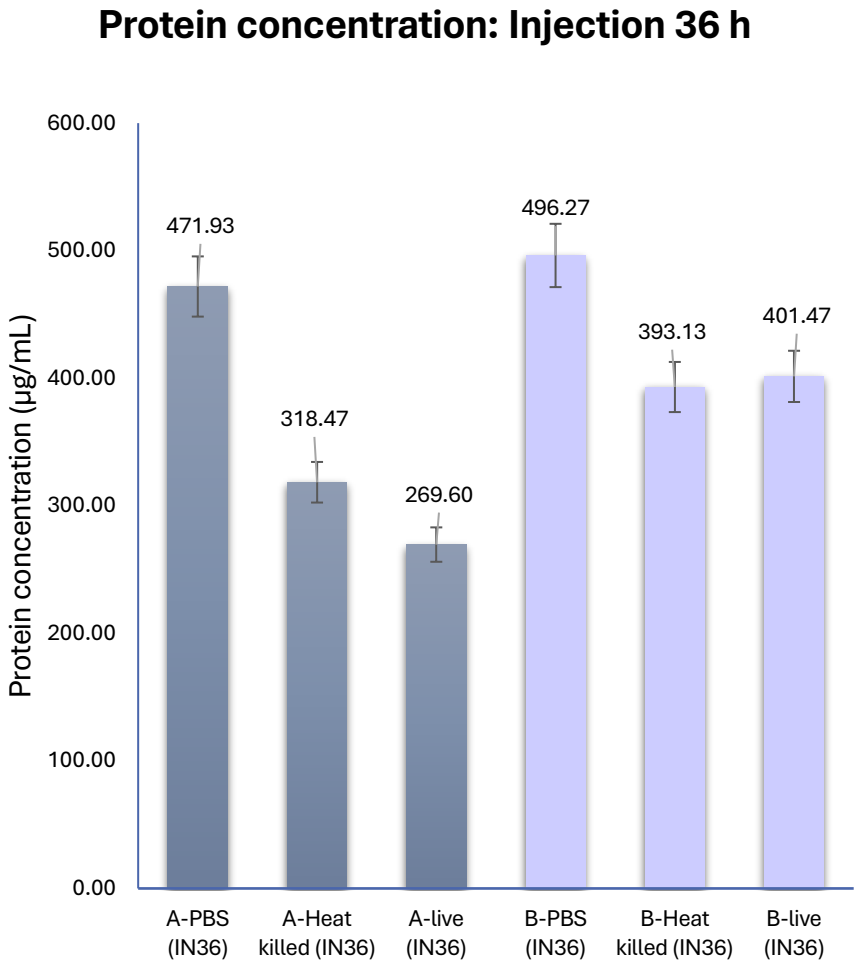


37 °C, for 18 h

Measured the inhibition zone

Ampicillin 10 µg (positive control), DI water (negative control)

Results: BCA analysis for total protein quantification

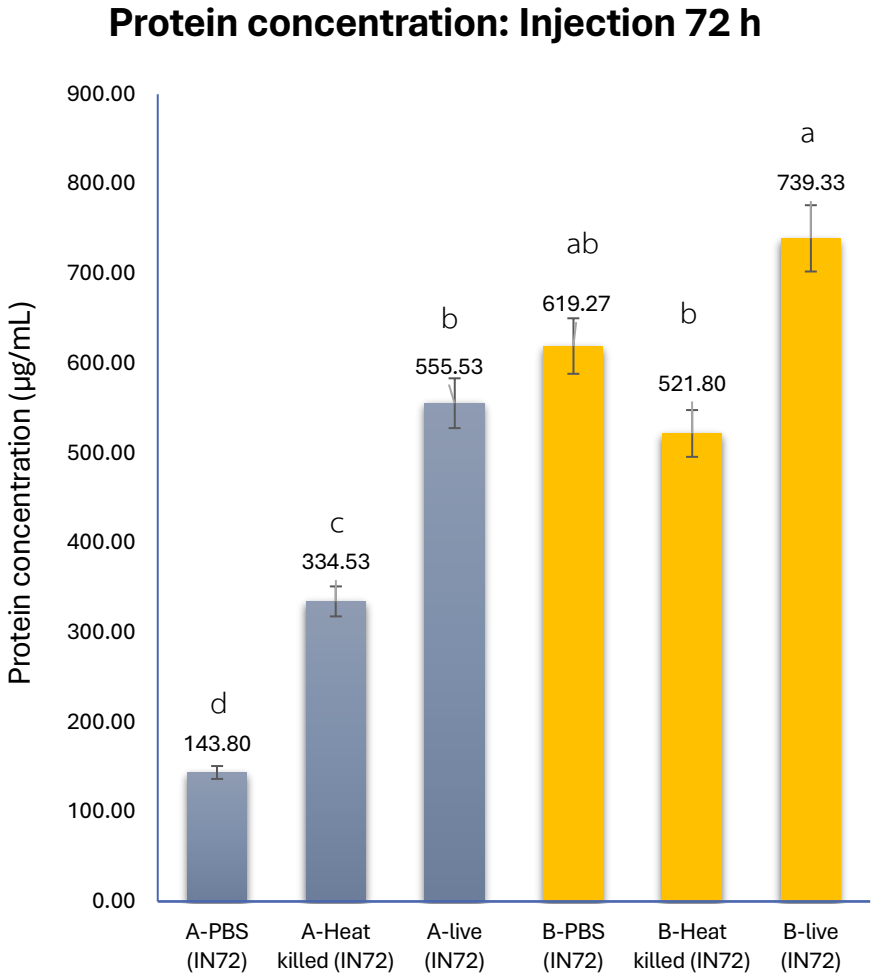


(p-value = 0.078)

The protein concentrations ranged from 269.60 to 496.27 µg/ml

No significant differences in protein concentration were observed between feed A and feed B

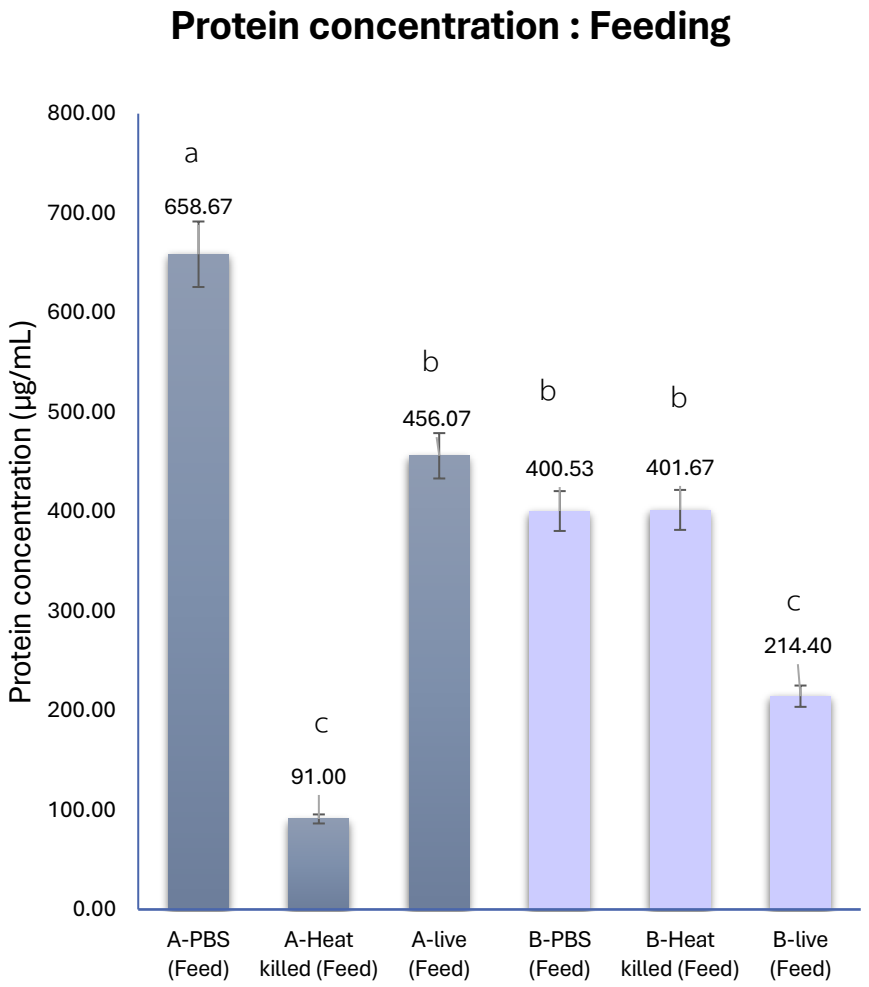
Results: BCA analysis for total protein quantification



Feed B exhibited protein concentration, ranging from 521.80 to 739.33 µg/ml.

Feed B exhibited higher protein concentration, however, within feed B, the heat-killed cells condition did not differ significantly from the A- live cells condition

Results: BCA analysis for total protein quantification



Feed A under the PBS condition showed the highest protein concentration.
Feed B under the PBS and heat-killed cells condition, as well as Feed A under the live cells condition shows no significant differences.

Results: BCA analysis for total protein quantification

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	A-PBS	500	471.93	0.09
	A-Heat killed	500	318.47	0.06
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

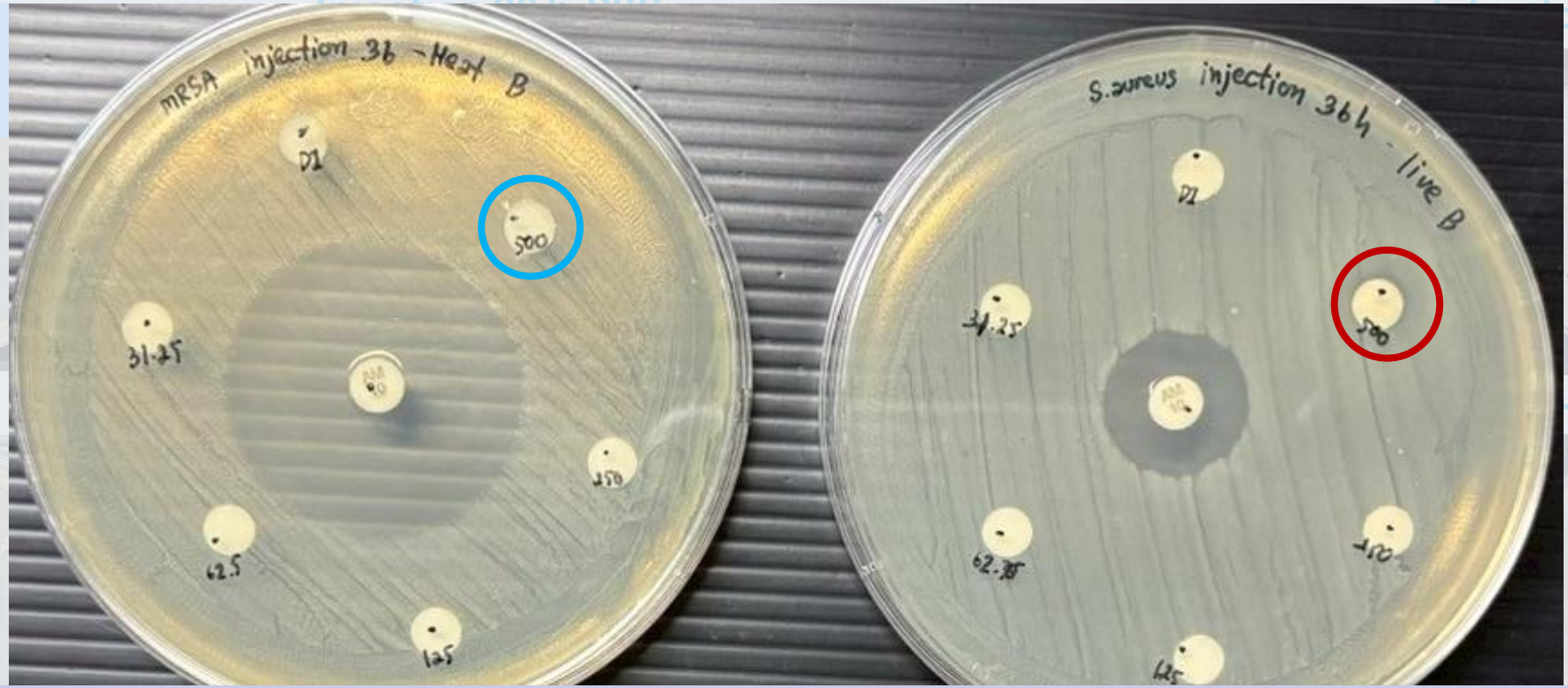
*A= Feed A, B= Feed B

these results suggest that the crude AMP extracts may contain a high proportion of other components, such as cell fragments, cell debris, and residual solutions

Moreover, protein content was calculated from 500 mg/ml crude AMP extracts ranging from 0.02% to 0.15%

Results: Disc diffusion assay

Injection 36 h Feed B



A turbid inhibition zone against *S. aureus* was observed in all conditions
Crude AMP concentration 500 mg/ml
Furthermore, inhibition against MRSA 1-1463 was detected in
The Feed B heat-killed cell, producing an inhibition zone.

Previous progression

Extracted AMPs from BSF larvae

Increase crude AMPs

1. Immunization *via* hemolymph

2. Immunization *via* feeding

Under conditions:

1. Control (PBS buffer)
2. Live cells
3. Heat kill

crude AMPs

The crude AMPs were dissolved

Antibacterial activity test

(Part II)

The characterization of AMPs

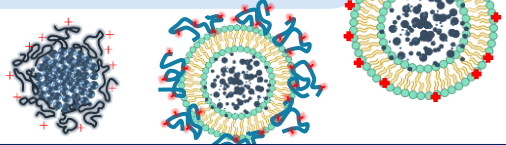
(Part III)

3

Immunization:

Immunization *via* hemolymph and feeding

Under conditions: F1, F2, and F3 (from Nanotech)



Extracted AMPs

Increase the amount of crude AMPs extract collected

Progressions

Immunization of BSF larvae

Hemolymph vs **Feeding**

Hemolymph	Feeding
Live cells	Live cells
Heat kill	Heat kill
F1	F1
F2	F2
F3	F3
Control	Control

Part I

AMP extraction from immunized larvae

↓

Determined the amount of AMP from **Hemolymph vs Feeding**

Part II

Antibacterial activity of AMP from immunized larvae against chicken pathogen

↓

Disc diffusion
MIC
MBC

Part III

Characterization of AMP from immunized larvae

↓

Column separation

↓

Mass spectra photometry

Part IV

Application of immunized BSF larvae as supplement in chicken feed

Bacterial nanoparticles

F1: Formula 1 killed bacteria coated with chitosan
F2: Formula 2 killed bacteria encapsulated in cationic Lipid nanoparticles
F3: Formula 3 killed bacteria encapsulated in cationic Lipid nanoparticles coated with fucoidan

Progressions

Methods: 1. immunization of BSF larvae

Under conditions

PBS (control)
Bacterial nanoparticles
- **F1: Formula 1 killed bacteria coated with chitosan**
- **F3: Formula 3 killed bacteria encapsulated in cationic Lipid nanoparticles coated with fucoidan**

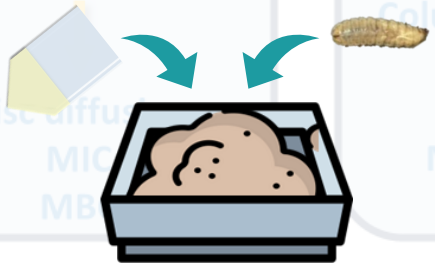
1 The hemolymph injection

(36h, and 72h)

The hemolymph in the third abdominal segment

harvest

2 The feeding



1. **Record the post-feeding weight**
2. **Count the number of dead larvae (mortality rate)**
3. **The enumeration of *E. coli* and *S. aureus* present in the feed**
   - Petrifilm™Staph Express Count Plate
   - Petrifilm™ *E. coli*/Coliform Count Plate; Neogen.

Results: Observation of immunization of BSF larvae

Table 1: Wet weight and larval mortality 36 h post-injection

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

Fig. 1: Rate of weight change under injection 72 h

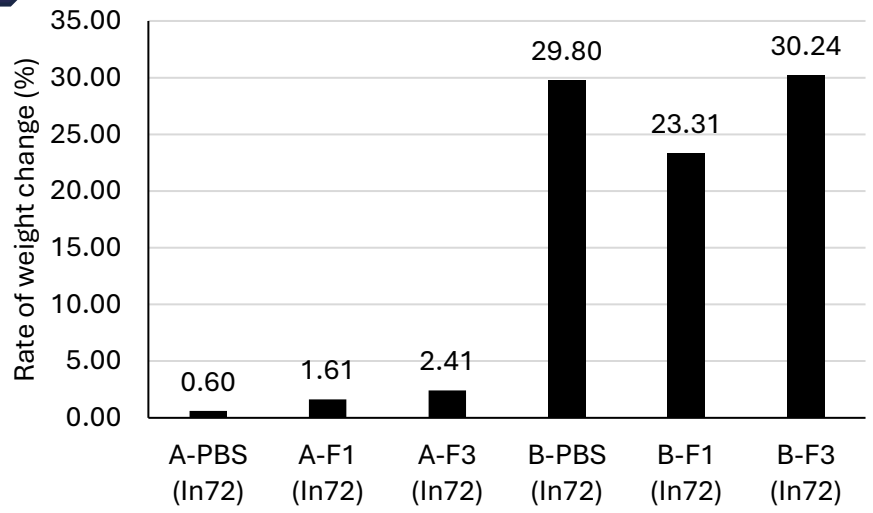
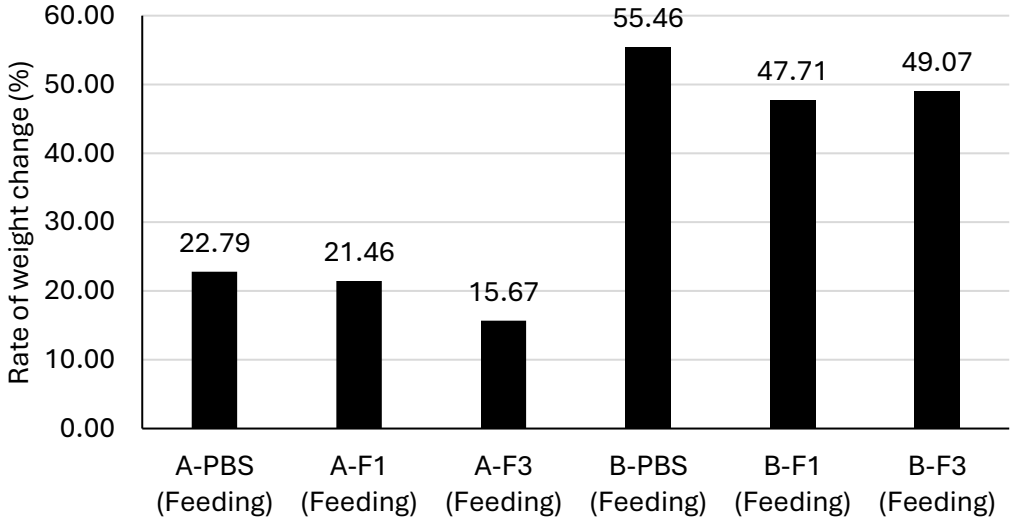
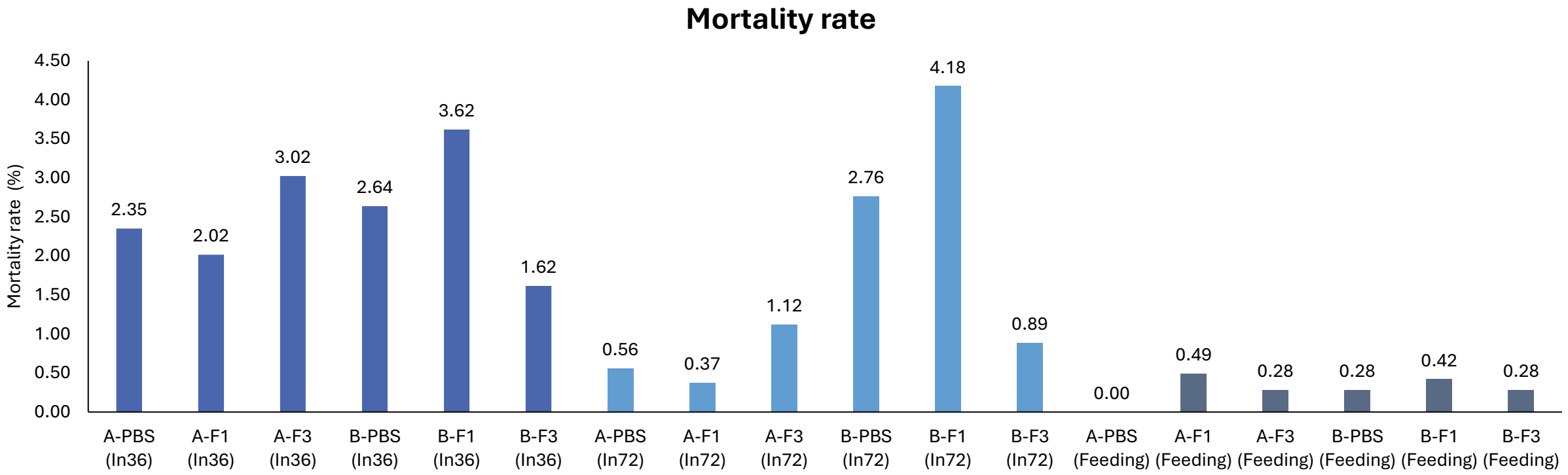


Fig. 2: Rate of weight change under Feeding 72 h



Results: Observation of immunization of BSF larvae

Fig. 3: The mortality rate under different experimental conditions

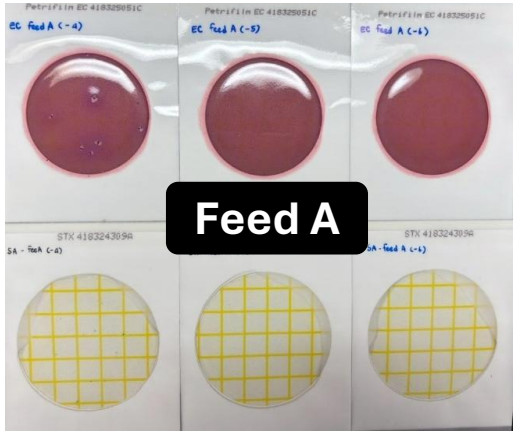


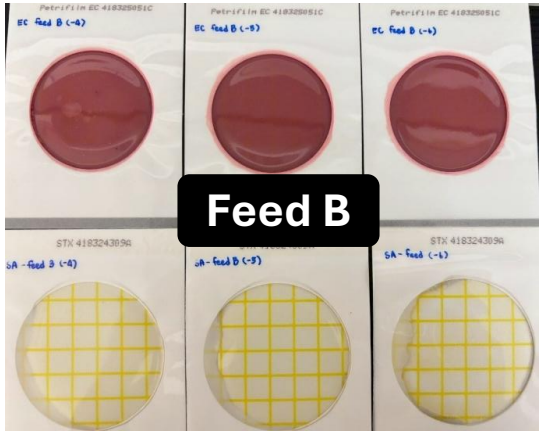
Therefore, immunization of BSF larvae *via* feeding methods can result in greater weight gain, indicating a higher survival rate.

Results: Bacterial culture from feeding

The enumeration of *E. coli* and *S. aureus* present in the feed

Fig. 4: Results of Petrifilm™ Count Plate on day 0



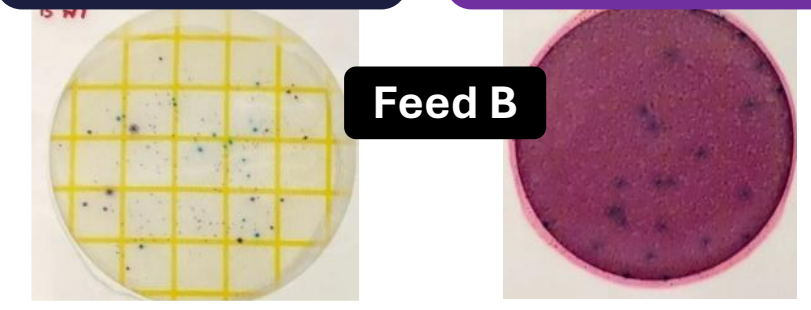


- *No S. aureus/E. coli* on day 0 in both feeds.
- Feed B showed higher bacterial counts on day 3.

Fig. 5: Results of Petrifilm™ Count Plate on day 3

S. aureus	*E.coli* 6.0×10^4 CFU/ml

S. aureus 1.04×10^6 CFU/ml	*E.coli* 2.5×10^5 CFU/ml



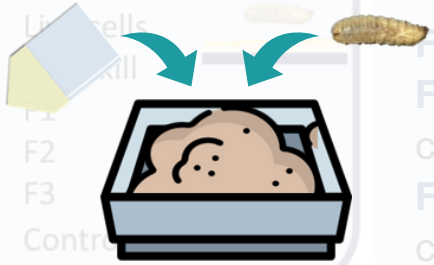
Lyophilized BSF larvae

extracted with acidified methanol
methanol: water: acetic acid (90:9:1 v/v/v)

crude AMPs

The hemolymph injection

The feeding



harvest

Part II

Antibacterial activity of AMP from immunized larvae against chicken pathogen

↓

Disc diffusion
MIC
MBC

Part III

Characterization of AMP from immunized larvae

↓

Column separation

↓

Mass spectra photometry

Part IV

Application of immunized BSF larvae as supplement in chicken feed

Under conditions

PBS (control)

Bacterial nanoparticles

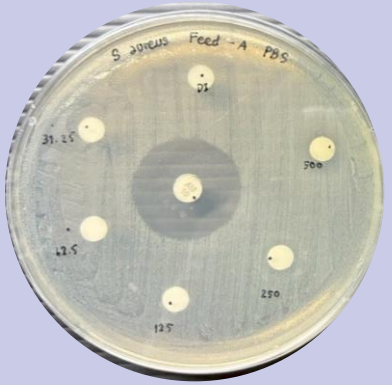
- **F1**
- **F3**

Conclusions

- **Protein content: Crude AMP extracts contained 0.02–0.15% total protein (BCA assay)**
- **Antibacterial activity: Crude AMP extracts inhibited *S. aureus*.**
- **Immunization-feeding method increased wet weight and reduced mortality.**

The disc diffusion assay

500 mg/ml crude AMP a diffuse inhibition zone against *S. aureus* ranging from 10.67 to 7.67 mm



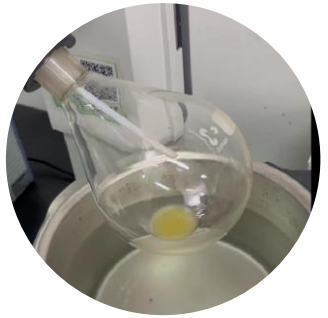
- The largest inhibition zone: Feed B PBS and heat-killed cells under the 36h injection
- The inhibition against MRSA 1-1463 was detected in the Feed B heat-killed cell

Next step:

Lyophilized BSF larvae

Under conditions:

1. Control (PBS buffer)
2. F1
3. F3



extracted with acidified methanol

Antibacterial activity test

1. Disc diffusion
2. MIC
3. MBC

Thesis plan :

Plane/time	1st year		2nd year		3rd year	
	Semester 1	Semester 2	Semester 1	Semester 2	Semester 1	Semester 2
1. Literature review						
2. Immunization of encapsulated nanoparticles-bacteria and bacterial cells *via* hemolymph BSF larvae		✓				
3. Immunization of encapsulated nanoparticles-bacteria and bacterial cells *via* feeding		✓				
4. Extracted crude AMPs from BSF larvae that are immunized *via* hemolymph and feeding		✓				
5. QE examination						
6. Antibacterial activity testing of AMPs extraction						
7. To investigate structure and functions of AMPs from BSF larvae						
8. Proposal Examination						
9. To evaluate BSF larvae's capability and potential application in chicken feed						
10. Data Analysis						
11. Thesis Defense Examination						

Done
Pending
Further

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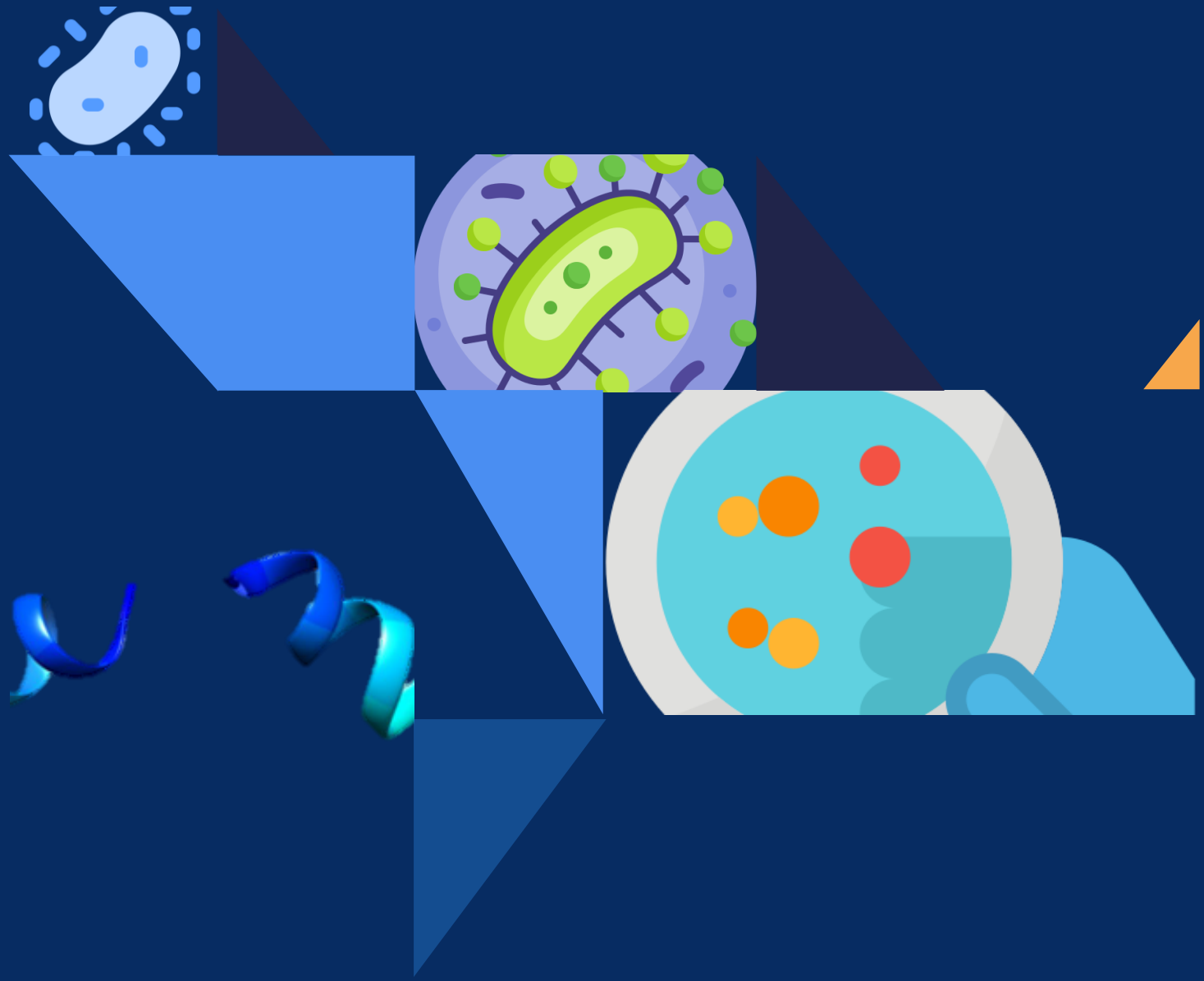

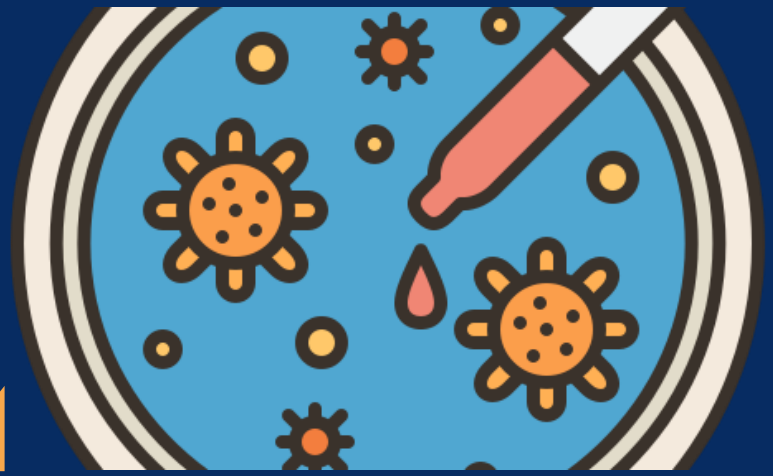

Thank you for your kind attention