



Research article

Effect of artificial diet supplemented with antibiotics and antimicrobial peptides on silkworm (*Bombyx mori* L.)

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Article Info

Article history:

Received 25 April 2025

Revised 23 September 2025

Accepted 23 October 2025

Available online 21 November 2025

Keywords:

Antibiotics,
Antimicrobial peptides,
Artificial diet,
Silkworms,
Survival rates

Abstract

Importance of the work: Understanding the effects of antibiotics and antimicrobial peptides (AMPs) in artificial diets is crucial for silkworm growth, gut microbiota and disease prevention.

Objectives: To examine the effects of antibiotics and AMPs on silkworm growth, development and survival during the first-to-third instars.

Materials and Methods: Silkworms (*Bombyx mori* L.) were reared under controlled conditions and fed artificial diets containing antibiotics (amoxicillin) and AMPs. The diets were prepared using mulberry leaf powder, soy protein, vitamins and other ingredients. Biological parameters (survival rate, larval weight and development) were recorded and analyzed using Duncan's test for significance. Four artificial diet formulations were evaluated: control (no antibiotics or AMPs), antibiotics (4%) and AMPs (0.2% and 0.4%).

Results: Survival rates during the third instar differed significantly ($p < 0.01$): control (45.98%), antibiotics (38.16%), AMP 0.2% (21.74%) and AMP 0.4% (3.55%). In the second instar, the control and antibiotic groups had the highest weights (0.09 ± 0.01 g), significantly ($p < 0.01$) higher than AMP 0.2% (0.07 ± 0.01 g) and AMP 0.4% (0.06 ± 0.01 g). In the third instar, there was a significant ($p < 0.05$) difference only between the control group (0.28 ± 0.02 g) and the AMP 0.4% (T4, 0.09 ± 0.03 g). Based on these results, an antibiotic-free and AMP-free diet was beneficial for silkworm health and growth.

Main finding: An antibiotic-free and AMP-free artificial diet supported optimal silkworm growth by preserving gut microbiota integrity. This novel approach eliminated the need for antibiotics, with important implications in advancing animal dietary research and the silk industry.

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<https://doi.org/10.34044/j.anres.2025.59.6.11>

Introduction

Silkworms (*Bombyx mori*) are economically important insects extensively reared for silk production (Chen et al., 2018). Traditionally, they rely on mulberry leaves, which provide essential nutrients for optimal growth and silk yield (Nath et al., 2025). However, mulberry cultivation faces major challenges, including limited land availability, seasonal fluctuations and rising agricultural costs. To address these constraints, artificial diets have been developed as an alternative, particularly in regions where mulberry leaves are scarce (Sushmitha et al., 2025). While these diets offer a viable solution, they often result in suboptimal growth, delayed development and reduced disease resistance in silkworms (Qin et al., 2022).

Commonly, the adverse effects of artificial diets are mitigated by incorporating antibiotics to prevent microbial infections, with several studies reporting improvements in cocoon production and silkworm health (Bhat and Sharada, 1956; Mahdi et al., 2017; Rafiq et al., 2020). However, the overuse of antibiotics raises concerns regarding antimicrobial resistance, environmental contamination and sustainability, as prolonged use can further promote resistant bacterial strains and complicate disease management in sericulture and beyond (Mahmoud et al., 2012). The gut microbiota of silkworms play a crucial role in digestion, nutrient absorption and immunity; however, antibiotics can disrupt this delicate balance, resulting in digestive dysfunction, malnutrition and greater susceptibility to infections (Mahdi et al., 2017). Although these risks highlight the urgent need for sustainable alternatives to antibiotics, only limited studies have evaluated potential substitutes and their effects on silkworm growth, survival and gut microbiota composition, constraining the advancement of sustainable, antibiotic-free sericulture practices.

Antimicrobial peptides (AMPs) have gained attention as promising candidates to replace conventional antibiotics (Ji et al., 2024). AMPs are naturally occurring short peptides found in a variety of organisms, including insects, plants and mammals and are known for their potent broad-spectrum antimicrobial activity with a low risk of inducing resistance (Nesa et al., 2020). Their primary mechanism involves the disruption of microbial cell membranes, leading to rapid pathogen elimination. In insects, AMPs, such as defensins, cecropins and attacins, play critical roles in maintaining gut homeostasis and protecting against microbial infections (Marra et al., 2021; Guo et al., 2024; Kariyanna and Nelluri, 2024; Lachat et al., 2024; Liang et al., 2024; Sekhavati, 2024).

Among these AMPs, cecropin AD—a synthetic chimeric peptide derived from cecropin A and D—displays stronger antimicrobial activity than its parental peptides (Christensen et al., 1988; Chen et al., 2009). In livestock, cecropins have been shown to suppress harmful *Escherichia coli* while promoting beneficial *Lactobacilli*, thereby enhancing gut health and immunity (Lachat et al., 2024). In addition, they have been reported to improve immune function and support intestinal balance across multiple animal species (Wen and He, 2012; Han and Shepherd, 2023; Ouyang et al., 2024; Li et al., 2025). In pigs and broilers, cecropin AD supplementation further reduced intestinal pathogens, improved gut morphology and enhanced growth performance (Wen and He, 2012; Peng et al., 2014). Another AMP of interest is plectasin, a defensin that has strong antimicrobial activity against Gram-positive bacteria, including resistant strains (Mygind et al., 2005). Despite these advances, there has been little published information regarding the effects of both cecropin AD and plectasin on silkworms.

Research on AMPs in artificial silkworm diets remains limited and systematic evaluation—particularly of Cecropin AD and Plectasin in *Bombyx mori*—is lacking. This study aimed to provide a novel, systematic comparison of antibiotics and AMPs in artificial diets during the first-to-third instar stages, which represent the most critical phases for dietary adaptation and larval survival (Shu et al., 2023; Wang et al., 2023). The hypothesis was that AMPs could replace antibiotics by enhancing growth, survival and gut microbial stability, without the associated risks of antibiotic use. The objective was to investigate and compare the effects of antibiotics and AMPs on silkworm growth and development, thereby evaluating their feasibility as sustainable alternatives for sericulture.

Materials and Methods

Silkworm rearing

The silkworms belonged to a polyvoltine strain of *B. mori* L., specifically the Nanglai variety—a local Thai strain commonly used in sericulture under tropical conditions (Supaporn and Terenius, 2020). This strain was chosen for its suitability to the tropical climate and resilience under local rearing conditions. Silkworm eggs were obtained from Surin Province, Thailand and subsequently reared in the silkworm rearing room of the Biochemistry Laboratory, Department of Biochemistry, Kasetsart University, Bangkok, Thailand. Newly hatched larvae were fed fresh mulberry leaves until cocoon

formation and pupation. Once the cocoons were formed, they were carefully opened to determine the gender of each pupa. Then, the pupae developed into adult moths, which mated and produced eggs for subsequent research.

The experiment was conducted under controlled environmental conditions at $25 \pm 2^\circ\text{C}$ with a relative humidity of $75 \pm 6\%$. The larvae were housed in plastic containers of varying sizes, depending on their developmental stage and density. Bed cleaning was performed once at the end of the molting stage during the first, second and third instars. Each treatment contained 180 larvae per replicate ($n = 3$ replicates), giving a total sample size of 540 larvae per treatment and 2,160 larvae across all treatments.

Artificial diet preparation

Artificial diet formulations for the first-to-third instar larvae were divided into four groups: a control diet (without antibiotics or AMPs); a diet supplemented with antibiotics (final concentration of amoxicillin: 4%); and diets containing antimicrobial AMPs at 0.2% and 0.4% concentrations. Amoxicillin was used as the antibiotic, while the AMPs were cecropin AD (purchased from Hinabiotech, China), targeting Gram-negative pathogens, with the plectasin (purchased from Hinabiotech, China) targeting Gram-positive pathogens. In the AMP-supplemented groups, the 0.2% diet contained 0.1% cecropin AD and 0.1% plectasin,

while the 0.4% diet contained 0.2% cecropin AD and 0.2% plectasin. The four experimental groups were: the control group; the antibiotic group; the 0.2% AMPs group; and the 0.4% AMPs group, as summarized in Table 1.

The base artificial diet (Table 1) was composed of mulberry leaf powder, soybean protein isolate, soybean protein, corn starch, soybean fiber, ascorbic acid, citric acid, sorbic acid, Wesson's salt mixture, choline chloride, methylparaben, beta-sitosterol, sucrose, soybean oil, agar and a vitamin mixture containing choline chloride, niacin, calcium pantothenate, thiamine (B1), riboflavin (B2), pyridoxine (B6), folic acid, inositol, biotin and vitamin B12. To prepare the diet, the ingredients were blended using a HW-CH2 blender (House Worth; Bangkok, Thailand). Agar (Pearl Mermaid agar powder; Bangkok, Thailand) was dissolved in water and heated in a microwave oven (R-250; Sharp; Bangkok, Thailand) for approximately 2 min until fully melted. Then, the heated mixture was combined with the mulberry leaf powder, soybean protein isolate, soybean protein, corn starch, soybean fiber and sucrose, continuously blended and left to cool for approximately 10 min. After cooling, the vitamins, minerals, or antibiotics were added according to each formulation and mixed thoroughly. Each prepared diet was allowed to cool to room temperature, then stored at 4°C until use. Before feeding, the diet was cut into appropriately sized pieces for each instar stage. Each treatment was replicated three times and silkworms were fed once daily.

Table 1 Composition of artificial diets for rearing silkworm larvae in 1st–3rd instar stages.

Ingredient	Artificial diet (g/100 g diet)			
	Control group	Antibiotic group	0.2% AMPs group	0.4% AMPs group
Mulberry leaf powder	3.14	2.93	3.14	3.13
Isolate soybean protein	3.30	3.08	3.29	3.29
Soybean protein	8.97	8.37	8.95	8.93
Corn starch	1.16	1.09	1.16	1.16
Soybean fiber	3.62	3.37	3.61	3.60
Ascorbic acid	0.69	0.65	0.69	0.69
Citric acid	0.31	0.29	0.31	0.31
Sorbic acid	0.16	0.15	0.16	0.16
Agar	2.20	2.05	2.20	2.19
Wesson's salt mixture	1.10	1.03	1.10	1.10
Choline chloride	0.06	0.06	0.06	0.06
Vitamin mixture	2.38	2.22	2.38	2.37
Methylparaben	0.22	0.21	0.22	0.22
Amoxicillin (0.4%)	-	2.01	-	-
AMPs	-	-	0.06	0.13
Beta-sitosterol	0.13	0.12	0.13	0.13
Sucrose	1.82	1.70	1.82	1.82
Soybean oil	0.72	0.67	0.72	0.72
Water	70	70	70	70
Total	100	100	100	100

AMPs = antimicrobial peptides.

Evaluation of biological parameters

Various biological parameters were recorded to evaluate the impact of the artificial diet on silkworm growth: survival rates at different larval stages, fresh larval weight and developmental duration of each larval stage (larval rearing duration: 1st instar = 7–8 d; 2nd instar = 4–5 d; 3rd instar = 6–7 d).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) to assess variance among groups. Duncan's multiple range test was used for post-hoc comparisons to identify significant differences between treatments at the $p < 0.05$ level. For datasets that did not meet the assumptions for ANOVA, differences between treatments were assessed using the Kruskal-Wallis rank-sum test, followed by pairwise comparisons with Dunn's test.

Ethics statement

All procedures involving animals were approved by the Institute of Animals for Scientific Purposes Development (Approval number: U1-01736-2558) and were performed in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee (IACUC), Kasetsart University Bangkok, Thailand. The IACUC oversees animal research ethics to promote ethical standards in the use of animals for scientific purposes, following guidelines and criteria related to the care and use of animals for research purposes.

Results

There were no significant differences in survival rates among treatments during the first and second instar stages. However, a highly significant ($p < 0.01$) divergence emerged during the third instar, wherein AMP supplementation elicited a clear, dose-dependent reduction in larval survival (Fig. 1). Pairwise comparisons indicated that the control group had significantly higher survival rates than both the AMP-supplemented groups, with the 0.4% AMP treatment producing the most substantial decline. These findings indicated that AMP supplementation, particularly at elevated concentrations, detrimentally affected larval viability during the third instar.

Larval weight and growth performance based on larval body weights varied significantly among treatments in both the second and third instars (Fig. 2). AMP supplementation led to a concentration-dependent decrease in growth, with the 0.4% AMP group having the most pronounced suppression. In contrast, larvae reared on the control and antibiotic-supplemented diets had significantly higher body weights, reflecting improved growth performance relative to AMP-fed cohorts. These findings underscored the inhibitory effects of AMPs on silkworm growth, with higher concentrations producing greater impairment. Visual assessments (Fig. 3) further corroborated these findings, with the AMP-fed larvae having visibly stunted growth compared to those raised on the control or antibiotic diets. The growth-retarding effect of AMP supplementation, particularly at 0.4%, was evident in both the visual and quantitative data, reinforcing the conclusion that AMPs adversely influenced silkworm development in a dose-dependent manner.

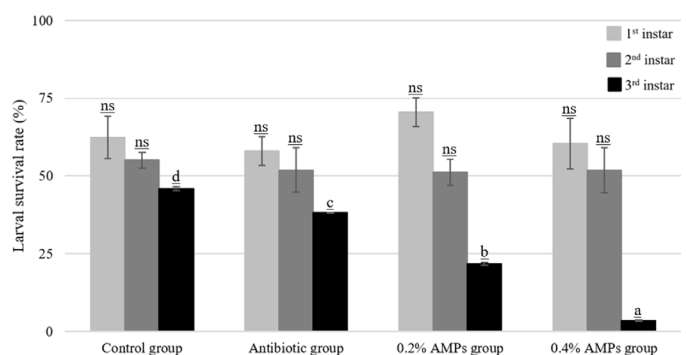


Fig. 1 Survival percentages of silkworms at the 1st–3rd instar stages fed four artificial diet formulas. Error bars represent $\pm$ SD. Different lowercase letters above the bars indicate significant differences ($p < 0.01$, Duncan's multiple-range test) among means within each stage; ns indicates non-significant difference. AMPs = antimicrobial peptide.

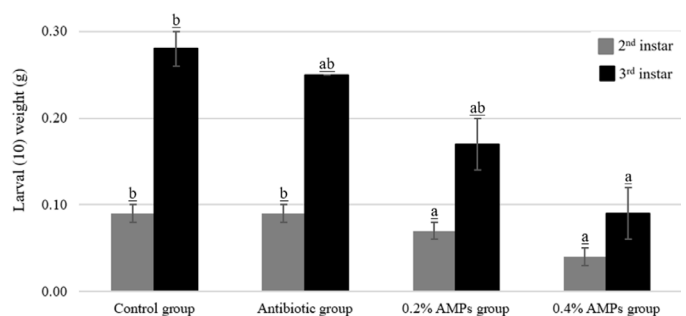


Fig. 2 Larval weight of silkworms at the 2nd and 3rd instar stages fed four artificial diet formulas. Error bars represent $\pm$ SD. Significant differences in the 2nd instar were determined using Duncan's multiple-range test ($p < 0.01$), while those in the 3rd instar were determined using Dunn's test ($p < 0.05$). Different lowercase letters within each instar stage indicate significant differences. AMPs = antimicrobial peptides.

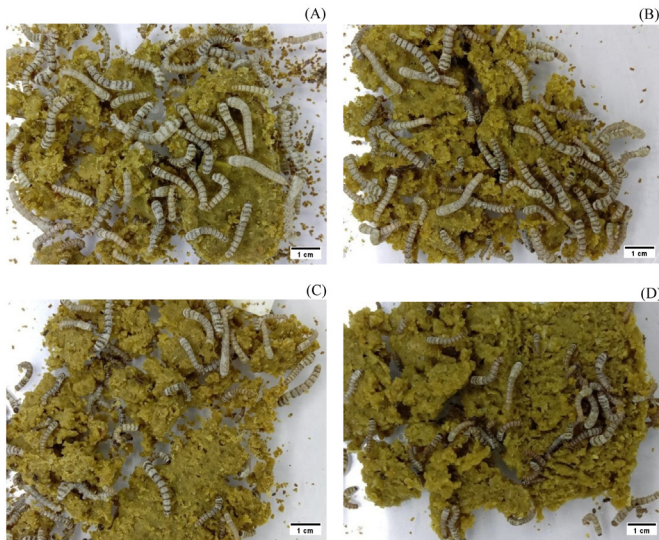


Fig. 3 Representative images of the 3rd instar larvae in different feeding treatments, highlighting differences in larval size: (A) control group; (B) antibiotic group; (C) 0.2% antimicrobial peptides (AMPs) group; (D) 0.4% AMPs group.

The developmental durations of each larval instar and the total larval period are summarized in Table 2. All three replicates produced identical values, yielding zero standard deviation and precluding statistical analysis. Minor treatment-related differences were observed in stage-specific durations, with the AMP supplementation tending to extend the second instar, while both the antibiotic and 0.4% AMP treatments prolonged the third instar. Despite these instar-level variations, the cumulative larval period remained constant at 18 d for most treatments, with only the 0.4% AMP group having a slight extension, reaching 19 d. These findings suggested that while antibiotics and AMPs may have altered the timing of individual instars, their overall impact on total developmental duration was minimal.

Table 2 Larval duration (mean $\pm$ SD, n = 3) of silkworms in 1st–3rd instar stages fed on four artificial diet formulas.

Treatment	Larval duration (d)			Total larval period (d)
	1 st Instar	2 nd Instar	3 rd Instar	
Control group	8 $\pm$ 0.00	4 $\pm$ 0.00	6 $\pm$ 0.00	18
Antibiotic group	7 $\pm$ 0.00	4 $\pm$ 0.00	7 $\pm$ 0.00	18
0.2% AMPs group	7 $\pm$ 0.00	5 $\pm$ 0.00	6 $\pm$ 0.00	18
0.4% AMPs group	7 $\pm$ 0.00	5 $\pm$ 0.00	7 $\pm$ 0.00	19

AMPs = antimicrobial peptides.

Discussion

The low survival rate, reduced weight and extended developmental period observed in the antibiotic-treated and the AMP-supplemented groups suggested that these treatments may have negatively impacted larval growth performance. Typically, with silkworms raised under standard conditions using mulberry leaves, the first-to-third instars last approximately 14 d (Sirimungkararat et al., 2010). In contrast, the larvae on the artificial diets in the current study took approximately 18–19 d, consistent with Sukhapinda et al. (1983). This delay could be attributed to the metabolic adjustments required for digesting and assimilating artificial feed, influenced by factors such as diet composition, digestibility, nutrient availability and environmental adaptation (Li et al., 2023).

Broderick et al. (2000) reported that lepidopteran larvae fed a combination of antibiotics displayed multiple health impairments. Specifically, exposure to a tetracycline mixture led to a 50% reduction in larval survival within the first 20 d post-hatching, with the surviving larvae weighing only one-tenth of those in the untreated control group. Similarly, Costa et al. (1997) examined the effects of various antibiotics on the growth, survival and reproduction of *Bemisia argentifolii* (whitefly) and found that antibiotics inhibiting bacterial protein synthesis, such as tetracycline and rifampicin, significantly impaired growth and development. However, antibiotics targeting bacterial cell walls or membranes, such as penicillin, ampicillin and lysozyme, had no significant effects on survival but delayed development and reduced the percentage of offspring emerging as adults. That study clearly demonstrated that antibiotic-free artificial diets, combined with strict hygiene and stabilizers (such as methylparaben), are better for silkworm development during the critical first to third instars than diets with antibiotics or AMPs. This novel finding offers practical insights for reducing antibiotic use in sericulture, addressing global antimicrobial resistance concerns.

These findings suggest that gut microbiota disruption caused by antibiotics and AMPs may be responsible for the reported declines in silkworm survival, growth and development. This highlights the critical role of maintaining a balanced gut microbiota for optimal silkworm health and development. The current results aligned with the other studies discussed above, indicating that gut microbial balance plays a crucial role in nutrient absorption, immune function and overall insect health.

The current study used amoxicillin as the antibiotic treatment. Amoxicillin, a moderate-spectrum antibiotic that is effective against both Gram-positive and Gram-negative bacteria (Rolinson, 1974), has been reported to enhance food consumption, utilization and digestibility in silkworm larvae, particularly at a 5% concentration (Mahdi et al., 2017). However, in the current study, 4% amoxicillin did not affect larval weight but reduced silkworm survival, differing from Mahdi et al. (2017), where the 5% concentration improved growth. This discrepancy may have been due to strain differences, as Mahdi et al. (2017) used the Bangladeshi Urboshi-1 strain, while the current study used the locally adapted Nanglai strain. Thus, genetic and physiological variation between strains can influence responses to antibiotics, underscoring the need to consider strain-specific effects in artificial diet evaluations.

Concerns remain regarding the potential development of antibiotic-resistant bacteria, which could pose major risks to silkworm health (Sokhibova et al. 2022) and broader ecological systems (Mondal et al. 2025). While short-term survival rates may improve with antibiotics, the long-term impacts of antibiotics on reproductive success and immune function remain a concern, as observed in another insect study (Dickel et al., 2016). The risk of antibiotic resistance requires careful evaluation. In addition, methylparaben, a common preservative in animal feed, has been added to the artificial diet to inhibit fungi, yeast and some bacteria, and consequently extending the product's shelf life (Brannen et al., 2001). Although it has antimicrobial properties, methylparaben is not a therapeutic antibiotic (Crovetto et al., 2017). Its inclusion in the diet appears sufficient to maintain microbial stability without additional antibiotics.

AMPs, such as cecropin AD and plectasin, have broad-spectrum activity (Mygind et al., 2005; Chen et al., 2009). However, the current findings suggested that even at low doses, AMPs may non-selectively disrupt both harmful and beneficial microbes, potentially impairing digestion, nutrient absorption and larval development.

One notable finding in the current study was that silkworms fed antibiotic-free artificial diets had the highest survival and weight gain, whereas those given amoxicillin or AMPs had reduced growth, likely due to gut microbiota disruption. Indiragandhi et al. (2011) noted that antibiotics could damage the gut epithelium, eliminate beneficial microbes and impair metabolism. Furthermore, even at low doses, antibiotics caused malformations, suggesting microbial imbalance affected digestion, nutrient absorption and overall development.

Koga et al. (2007) demonstrated the effects of antibiotic-induced bacterial disruption in aphids, showing that injecting ampicillin (1000 ng/mg) effectively eliminated *Serratia* without affecting aphid survival. However, when rifampicin (2–20 ng/mg) was used to eliminate *Buchnera*, some aphid strains lost their reproductive ability. Furthermore, aphids lacking both *Buchnera* and *Serratia* did not survive beyond the first generation. These findings highlighted the crucial role of gut microbial symbionts in host survival and reproduction. Several studies have confirmed the strong correlation between gut bacteria and host nutrition, with experimental evidence indicating that certain gut microbes synthesize essential nutrients (Tamas et al., 2002; Douglas, 2009; Dapa and Xavier, 2024). Lin et al. (2015) found that *Providencia* sp. and *Carnobacterium maltaromaticum* exhibited antibiotic resistance, making them difficult to eliminate from the gut microbiota of *P. xylostella*. This suggests that some bacteria possess inherent resistance mechanisms, allowing them to persist despite antibiotic exposure. Ignasiak and Maxwell (2018) reported that antibiotics reduced gut microbiome diversity, increasing susceptibility to opportunistic infections and antibiotic-resistant strains; in *Galleria mellonella*, exposure to oxytetracycline resulted in the loss of genes responsible for tetracycline resistance, making the microbiome more vulnerable to domination by resistant bacterial strains. Lin et al. (2015) demonstrated that antibiotics not only reduced bacterial abundance but also altered the microbial community dynamics, favoring *Carnobacterium* sp., *Providencia* sp. and *Enterococcus* sp., while eliminating previously dominant species such as *Staphylococcus* sp. Additionally, Gao et al. (2021) highlighted that gut microbiota disruption impaired digestion and physiological balance, reducing the host's ability to maintain homeostasis and nutrient absorption. Collectively, these findings emphasize the need for cautious antibiotic use in silkworm diets to avoid unintended negative effects on growth and survival.

Given the concerns surrounding antibiotic resistance and gut microbiota imbalances, alternative solutions are required. AMPs have evolved over millions of years and are highly conserved, suggesting a low risk of bacterial resistance (Gasco et al., 2020). The current study investigated cecropin AD and plectasin as potential antibiotic alternatives. However, based on the current results, AMP supplementation did not improve silkworm growth, while at higher concentrations, these AMPs even had a negative effect, suggesting that while they are designed to target harmful bacteria, they may also disrupt beneficial gut bacteria.

Regulation of antimicrobial peptide (AMP) activity has been reported in insects, such as *Drosophila melanogaster*, *Galleria mellonella*, *Rhodnius prolixus* and *Bactrocera dorsalis*, where it is mediated by the intestinal Immune Deficiency-Nuclear Factor κ B signaling pathway that balances pathogen clearance with the preservation of beneficial microbiota (Ryu et al., 2006; Zhai et al., 2018; Sarvari et al., 2020; Vieira et al., 2021). In *B. dorsalis*, region-specific AMP production in the foregut protects symbionts in the midgut (Zhou et al., 2024). When this regulation is absent, as with AMP supplementation in artificial diets, AMPs may act broadly and disrupt gut microbial balance. Although some AMPs have narrow specificity toward pathogenic bacteria, such as dipterin against *Providencia rettgeri* and drosocin against *Enterobacter cloacae*, no single AMP is effective universally, underscoring the importance of regulatory control and cooperative AMP action in insect immunity (Zhou et al., 2024). In *B. mori*, gut immunity is less characterized, but oral infection studies have revealed region-specific induction of AMPs, such as CecA1, gloverin and moricin, with stronger responses in the foregut and midgut than the hindgut (Wu et al., 2010). In addition, BmToll9-1, a toll-like receptor highly expressed in the midgut, positively regulates AMP production and antibacterial activity, underscoring the role of immune signaling in gut defense (Liu et al., 2024). Notably, local gut immunity in *B. mori* exhibits regional specificity. These findings emphasize that while AMPs play an essential role in insect gut immunity through tightly regulated and region-specific expression, their uncontrolled supplementation may disrupt microbial homeostasis and compromise host health. These beneficial microbes are essential for digestion and nutrient absorption and their elimination could negatively impact silkworm health and development. AMPs exert antibacterial activity by disrupting bacterial membranes or interfering with intracellular processes. While this mechanism is crucial in combating antibiotic-resistant bacteria (Gagat et al., 2024), their amphiphilic structure allows them to bind to microbial membranes and, potentially, affect eukaryotic cells, including gut microbiota (Mihaylova-Garnizova et al., 2024). The effectiveness of AMPs is influenced by their charge and hydrophobicity, which can be optimized to enhance selectivity for pathogenic bacteria, while minimizing effects on beneficial microbes (Gagat et al., 2024). However, modifications to increase antimicrobial activity may inadvertently raise toxicity to non-target organisms, including gut bacteria (He et al., 2024). Although AMPs have demonstrated enhanced antimicrobial potency, selectivity and killing kinetics toward specific bacterial targets, they still

exert unavoidable bactericidal effects on non-target bacteria. This limitation is likely due to the killing domain of the peptides not being redesigned or modified, thereby retaining the electrostatic interactions between the cationic AMP moieties and the anionic lipids of bacterial membranes. Such interactions result in non-receptor-mediated, indiscriminate bactericidal activity and consequently, current strategies have yet to achieve the development of truly target-specific antimicrobial agents (Xu et al., 2020). Notably, electrostatic forces have been shown to primarily drive the initial interaction of AMPs with bacterial membranes, further supporting the persistence of non-specific targeting despite efforts to enhance specificity (Ma et al., 2024). While AMPs hold promise as alternatives to conventional antibiotics, their broad-spectrum activity necessitates careful consideration of their impact on the entire microbiome (Veldsman et al., 2025).

Mastore et al. (2021) explored the use of silkworm (*B. mori*) plasma as a source of AMPs and found that while the plasma effectively inhibited the growth of Gram-negative bacteria, such as *Escherichia coli* and *Enterobacter cloacae*, and Gram-positive bacteria, such as *Micrococcus luteus* and *Bacillus subtilis*, *Staphylococcus aureus* was highly resistant. This finding highlights the potential challenge of bacterial resistance, even to silkworm-derived AMPs. Thus, the development of new, highly effective AMPs remains critical. The use of AMPs should be reconsidered unless they can specifically target harmful bacteria without disrupting beneficial gut microbes or inducing resistance. More research is needed to identify AMPs that are both effective and selective, ensuring silkworm health is not compromised. Furthermore, the current study did not involve challenging the larvae with actual pathogenic microorganisms. It would be valuable to investigate whether the addition of antibiotics or AMPs might have shown positive effects on survival and growth if the silkworms had been exposed to real pathogens. Including this consideration would provide a more comprehensive interpretation of the results and suggest directions for future research.

Conclusion

A comparative evaluation was undertaken of antibiotics and antimicrobial peptides (AMPs) as dietary supplements in artificial diets for *Bombyx mori* larvae. The findings revealed that AMP supplementation, particularly at higher concentrations, significantly reduced larval survival and growth during the critical third instar stage.

While antibiotic-supplemented and antibiotic-free diets supported superior larval development and body weight, the AMP-treated groups exhibited dose-dependent impairments in both growth and viability. Although minor variations in developmental timing were observed, particularly under high-dose AMP or antibiotic treatments, the overall larval duration remained largely unaffected. These findings underscore the potential risks associated with incorporating AMPs—especially at elevated concentrations—into silkworm diets, highlighting their limited suitability as direct substitutes for antibiotics under the tested conditions. Future research should focus on rationally designing and modifying AMPs to target pathogens while preserving beneficial symbionts. Approaches such as site-directed mutations, cyclization, *de novo* peptide design and AI-assisted modeling could improve their specificity, stability and safety. Optimizing formulations and dosages will be essential to support gut health and immunity without impairing larval growth. Ultimately, precision-engineered AMPs offer a sustainable alternative to antibiotics, reducing collateral damage to host microbiota and guiding microbial management in artificial silkworm rearing systems.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This research was funded by the National Research Council of Thailand (NRCT) under Grant no. B4301-00235-63. Ruenmai-Baimon Partnership Limited provided partial financial support. Ms Phonphan Watthanarat was supported by the Science Achievement Scholarship of Thailand (SAST). The Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand provided the facilities for silkworm cultivation.

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