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SHORT COMMUNICATION
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Chicken Meat Supplemented with Livestock Blood as an Alternative Protein Source in Artificial Pollen Diets Supports Hypopharyngeal Gland Development, Lifespan, and Gut Microbiome in Honey Bees (*Apis mellifera* L.)

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ABSTRACT

Honey bee colony health relies on high-quality pollen, but shortages necessitate effective artificial diets. This study formulated chicken meat-based diets supplemented with livestock blood to meet macronutrient needs and evaluated their effects on growth, hypopharyngeal gland (HPG) development, lifespan, and gut microbiota. Diets were analyzed for protein, fat, carbohydrate, fiber, moisture, ash, and energy. Nurse bees received chicken blood-based (CB), mixed pollen (MP), or syrup-only (Sr) treatments in controlled cages; outcomes included HPG acini size, lifespan, and 16S rRNA gut microbiome profiles. Proximate analyses revealed balanced profiles: protein 12.7–15.7%, fat 4.7–5.1%, carbohydrate 45–46%, fiber 2.1–2.6%, ash 1.6–2.6%, moisture 30–34%, and energy 252.9–293.6 kcal/100 g. CB and MP diets yielded larger HPG acini and longer lifespans than Sr controls. The gut microbiome maintained diversity across diets; CB and MP promoted beneficial taxa while reducing pathogens, unlike Sr, which lowered diversity and enriched Bacillus. Chicken meat- and blood-based diets match natural pollen in supporting honey bee survival, physiology, and gut microbial balance. These findings pioneer cost-effective, protein-rich alternatives for sustainable apiculture.

Keywords: Alternative protein, Bee health, Chicken meat diet, Honey bee nutrition, Livestock blood supplement, Nutritional diet.

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INTRODUCTION

Nutritional stress in honey bees caused by climate change has led beekeepers to provide supplemental diets in the form of artificial pollen diet. These diets may either contain natural pollen or be formulated without it (Mortensen et al., 2019; Noordyke & Ellis, 2021; Ansaloni et al., 2025). Commercial artificial pollen diets typically contain 10-40% protein, depending on the ingredients used and the in-tended nutritional benefits for honey bees (Ricigliano et al., 2022). Optimal artificial diets mimic natural pollen ~20-25% protein, 5-10% lipids, 30-50% carbohydrates (dry matter basis), supporting hypopharyngeal gland (HPG) development as a key nurse bee indicator and gut microbiota balance (Powell et al., 2023). In addition, some manufacturers include additives such as lipids or phytosterols to enhance palatability and stimulate feeding (Noordyke & Ellis, 2021). In situations where natural pollen resources are limited, beekeepers are encouraged to provide artificial pollen diets that are equally effective, or more effective, than natural pollen. Beekeepers may either purchase commercially available artificial pollen diets or prepare their own formulations using diverse plant, animal, and microbial-derived protein sources (Lamontagne-Drolet et al., 2019). Several artificial diets have been developed and adopted worldwide, including MegaBee® and Ultra Bee® (USA), and Global Patties® (Canada) (Lamontagne-Drolet et al., 2019; Ricigliano et al., 2022).

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Haydak and Tanquary first reported on the use of blood powder in artificial pollen substitutes for honey bees (Haydak et al., 1943). However, this ingredient has received limited attention in subsequent research, possibly due to fresh livestock blood's high-water content, which complicates processing for incorporation into artificial pollen patties (Duarte et al., 1999). Danmek et al. (2025) reported that hydrolyzed porcine blood, used as a component (not a sole ingredient) with soy-based components in artificial pollen diets which improved honey bee health and longevity. These findings suggest that the functional role of livestock blood in artificial pollen diets may depend more on its integration with an appropriate protein matrix than on the specific origin of the accompanying protein source. When combined with livestock blood, soy-based ingredients enable effective artificial pollen diets, suggesting animal-derived proteins as viable alternatives to plant sources. Chicken meat, with its high protein content and balanced amino acid profile meeting honey bee nutritional needs, has been explored as such an alternative (Chuttong et al., 2025). In addition, poultry farming offers rapid production rates and high yields, providing a consistent, scalable protein supply to meet rising consumer demand (Korver, 2023). Combined with livestock blood, chicken meat warrants investigation as a promising, readily available protein matrix for artificial pollen diets.

However, its application in honey bees has not yet been explored and therefore requires systematic evaluation under controlled laboratory conditions before being extended to field-scale applications. Importantly, any formulation intended for honey bees must provide an optimal nutritional profile that supports health and growth, as well as other key physiological and performance parameters, to ensure that artificial pollen diets effectively meet the biological and ecological requirements of honey bees (Manning, 2016; Ricigliano et al., 2022). Protein sufficiency supports HPG acini development, extends lifespan, and maintains core gut taxa including *Snodgrassella alvi* and *Bifidobacterium*, whereas some substitutes impair these functions and disease resistance (Paray et al., 2021). Honey bee colony health relies on high-quality pollen, but shortages necessitate effective artificial diets (Di Pasquale et al., 2013; Bryś et al., 2021; Braglia et al., 2025). However, the potential of chicken meat combined with livestock blood as a protein matrix for artificial pollen diets in honey bees has not yet been systematically investigated. Its application requires evaluation under controlled laboratory conditions before field-scale use in colonies, ensuring optimal nutritional profiles that support health, growth, HPG development, lifespan, and core gut taxa. The objective of this study was to evaluate the efficacy of chicken meat-based artificial pollen diets supplemented with different types of livestock blood, including chicken, pig, cow, and buffalo for honey bees. We hypothesize that diets formulated with chicken meat as the primary protein matrix and supplemented with livestock blood can effectively support honey bee growth, health, longevity, and gut microbiota, potentially matching or exceeding the performance of the diets containing proteins derived from natural pollen. This study may contribute to sustainable and efficient beekeeping practices during periods of natural pollen scarcity by providing a low-cost, high-quality artificial pollen diet based on readily available chicken meat and livestock blood, thereby reducing reliance on labor-intensive natural pollen collection.

MATERIALS & METHODS

Preparation of Artificial Pollen Diets

Skinless chicken breast meat and livestock blood derived from chicken, pig, cattle, and water buffalo were pretreated following the methodology of Danmek et al. (2025). Samples were incubated with 2.0% (w/w) commercial acid protease enzyme in heat-resistant transparent plastic bags at 55°C in a water bath for 180 minutes. Enzymatic reactions were terminated by boiling in hot water for 15 minutes.

The chicken meat-blood (CB) diets were formulated as follows: 33% (w/w) pretreated chicken meat, 20.9% (w/w) pretreated livestock blood, 10% (w/w) mixed pollen powder, 30% (w/w) sugar, 0.05% (w/w) vitamin mix, 0.05% (w/w) mineral mix, 2.0% (w/w) palm oil, 1.0% (w/w) linseed oil, 2.0% (w/w) rum (40% alcohol), and 1.0% (w/w) xanthan gum. All ingredients except rum were mixed, homogenized, and heated in a microwave oven at 800 W for 10 minutes to ensure microbial safety and gel formation while minimizing nutrient loss (Zhang et al., 2023). After cooling to room temperature, rum was added and mixed to ensure uniform incorporation, as prior heating cause's alcohol evaporation. Final mixtures were transferred to containers for experimental use.

Characterization of CB Diets

The proximate composition of the diets, including crude protein, crude fat, crude fiber, ash, carbohydrate, and energy content, was determined following the procedures outlined by the Association of Official Analytical Chemists (AOAC) (Latimer, 2016).

Preparation of Honey Bee Colonies

Honey bee colonies were obtained from the apiary at the Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Thailand (N 19°4′21.432″, E 99°53′3.6234″) in March 2025 and maintained according to standard protocols (Chuttong et al., 2025). Sealed brood frames were removed from the honey bee colonies and transferred to an insect growth chamber set at a temperature of 33 ± 1.0°C with a relative humidity of 60 ± 1.0% and maintained in darkness until pupae metamorphosed into emerged adult honey bees.

Honey Bee Lifespan

Preparation and maintenance were carried out according to standard protocols (Chuttong et al., 2025). Thirty (30) newly emerged adult worker bees were housed in 600 mL polypropylene boxes fitted with 40 ventilation holes (≤ 5 mm in diameter). Two syringes, one

filled with water and the other with a 50% (w/w) sucrose solution (Sr), were attached to the box lids and served as liquid feeders. Two grams (3) of each CB diet were placed in plastic containers inside the cages for honey bee consumption. A mixed pollen diet (MP) was prepared by combining mixed pollen with 50% (w/w) sucrose in a 2:1 ratio. Water, syrup, and diet were monitored and replenished every 3 days. Daily observations were conducted to assess the longevity and lifespan of honey bees in all treatments throughout throughout the 21-day experimental period. Any deceased honey bees were promptly removed from the cages.

Hypopharyngeal Gland (HPG) Development

Preparation and maintenance followed the methodology described in Chuttong et al. (2025). On day 7, five honey bee samples from each treatment were collected to evaluate hypopharyngeal gland (HPG) acini development. The glands were dissected and placed in plastic petri dishes containing droplets of normal saline solution maintained at 25 °C. Micrographs were taken using an Olympus BX53 digital upright microscope (Olympus Corporation, Tokyo, Japan) equipped with an IMTcamCCD5 PLUS camera (IMT i-Solution, Inc., Vancouver, BC, Canada). For each HPG-acini, the diameters of 10 randomly selected HPG-acini with clearly defined borders were measured in pixels and converted to millimeters, with four replicates per treatment. The average values were used for statistical analysis.

DNA Sample Preparation for Metagenomic Sequencing of Honey Bee Gut

Metagenomic analysis was conducted on genomic DNA extracted from honey bee hindgut samples to preserve native microbial community composition. Hindguts were dissected and pooled to obtain sufficient biomass for DNA extraction. Because gut size and content varied among individuals, the number of hindguts per pooled sample was not fixed (ranging from 5-10 guts per sample), and pooling was performed based on obtaining sufficient material for downstream analysis. Technical triplicates of each pooled sample were obtained. Approximately 250 mg of each sample was processed using either the DNeasy PowerSoil® Pro Kit or the DNeasy PowerFecal® Pro Kit (QIAGEN) according to the manufacturer's protocol. Samples were incubated at 70°C for 20 min to enhance cell lysis, followed by mechanical disruption via bead beating. DNA integrity was assessed by agarose gel electrophoresis. Purity was evaluated spectrophotometrically using A260/A280 and A260/A230 ratios. DNA concentration was quantified using a Qubit 4 Fluorometer (Thermo Fisher Scientific) with the dsDNA High Sensitivity Assay Kit.

Metagenomic Library Preparation

The bacterial 16S rRNA gene was amplified using the UltraRun LongRange PCR Kit (QIAGEN). Barcoding was incorporated during amplification using the Rapid Sequencing DNA-16S Barcoding Kit 24 V14 (SQK-16S114.24, Oxford Nanopore Technologies). Barcoded amplicons were pooled and purified with AMPure XP beads to remove residual primers and PCR byproducts. DNA concentration was also measured using the Qubit Fluorometer prior to adapter ligation. Sequencing adapters and buffers were added based on the manufacturer's instructions.

Nanopore Sequencing and Data Analysis

Sequencing was performed on a MinION Mk1D device (Oxford Nanopore Technologies). Flow cells were equilibrated to room temperature before loading, and sequencing runs were monitored using MinKNOW™ software. Raw signal data were basecalled to generate FASTQ files. A total of 21 pooled gut samples yielded barcode-assigned reads and were included in downstream analysis. Sequencing depth ranged from 20,467 to 37,178 reads per sample (mean: ~25,687 reads), with 539,424 total reads across all samples. Taxonomic profiling was conducted using the EPI2ME 16S workflow for genus-level classification and the EPI2ME Metagenomics workflow for species-level identification and relative abundance estimation. Downstream microbial community analyses, including alpha diversity, beta diversity ordination (PCoA), and statistical comparisons, were performed using MicrobiomeAnalyst2.0 (accessed in December, 2025).

Statistical Analysis

Data with normal distribution were analyzed using one-way ANOVA, followed by post-hoc Tukey's test, with a significance level set at $P < 0.05$, conducted using SPSS version 27.0 (IBM Co., Armonk, NY, USA). Kaplan-Meier survival analysis was employed to evaluate the impact of diets on the lifespan of honey bees. The statistical significance of differences in time distributions between groups was assessed.

RESULTS & DISCUSSION

Proximate Analysis

Early research established livestock blood meal as a viable pollen substitute (Haydak et al., 1943). Building on this, recent studies confirm animal-derived proteins enhance nurse bee physiology, particularly hypopharyngeal gland development (Danmek et al., 2025; Chuttong et al., 2025). In this study, chicken meat-blood (CB) diets were formulated to provide 12-17% protein, 3-5% fat, and ~45% carbohydrate, matching honey bee nutritional requirements (Manning, 2016; Noordyke & Ellis, 2021; Ricigliano et al., 2022). Proximate composition varied significantly among treatments (Table 1). The mixed pollen diet (MP) had the highest protein ($P = 0.009$). Among CB diets, pork blood (CB-Po) and cow blood (CB-Co) showed elevated protein and energy, while chicken blood (CB-Ch) and buffalo blood (CB-Bu) had lower values. All CB diets exceeded MP fat content ($P < 0.001$). Energy was comparable to MP in CB-Po and CB-Co ($P = 0.046$). Ash, moisture, fiber, and carbohydrates showed minor variation. All diets fell within established nutritional ranges, confirming CB formulations adequately support honey bee growth and physiological function (Danmek et al., 2025).

Table 1: Proximate composition of chicken blood-based diets and control

Parameter (%)	Diets					P-value
	MP	CB-Ch	CB-Po	CB-Co	CB-Bu	
Moisture (%)	30.43 ± 2.0	33.78 ± 1.29	33.26 ± 2.57	32.81 ± 2.24	31.38 ± 2.20	0.334
Protein (%)	17.12 ± 0.12^{a}	12.88 ± 1.53^{b}	15.71 ± 1.47^{ab}	15.60 ± 1.19^{ab}	12.66 ± 1.76^{b}	0.009
Carbohydrate (%)	46.23 ± 1.44	45.27 ± 1.01	45.73 ± 1.76	46.23 ± 1.43	45.79 ± 2.07	0.937
Fat (%)	3.07 ± 0.21^{b}	4.83 ± 0.29^{a}	5.06 ± 0.19^{a}	5.07 ± 0.25^{a}	4.70 ± 0.44^{a}	<0.001
Fiber (%)	2.10 ± 0.30	2.47 ± 0.43	2.50 ± 0.44	2.63 ± 0.35	2.17 ± 0.42	0.440
Ash (%)	1.62 ± 0.25^{c}	1.81 ± 0.32^{bc}	2.51 ± 0.14^{a}	2.62 ± 0.17^{a}	2.27 ± 0.30^{ab}	0.002
Energy (kcal/100g)	273.98 ± 10.22^{ab}	252.86 ± 13.88^{b}	293.61 ± 19.15^{a}	283.48 ± 15.85^{ab}	266.75 ± 10.27^{ab}	0.046

Note: MP = mixed pollen diet, CB-Ch = chicken meat + chicken blood, CB-Po = CB + pork blood, CB-Co = CB + cow blood, CB-Bu = CB + buffalo blood. Data were analyzed using analysis of variance (ANOVA) and are presented as Mean ± SD. Means within a column with different superscripts are considered significantly different at P < 0.05, based on post hoc multiple comparisons using Tukey's b-test. Rows without superscript letters indicate no significant differences.

Hypopharyngeal Gland (HPG) Development and Lifespan

HPG acini development, a key indicator of honey bee growth, health, and physiological function (Ueno et al., 2015), differed significantly among dietary treatments. Honey bees fed the mixed pollen (MP) diet and all chicken meat-blood (CB) diets exhibited significantly larger HPG acini than protein-deficient syrup-only controls (Sr) ($P < 0.001$), demonstrating improved physiological status through protein supplementation (Jang et al., 2022; Mohamed et al., 2023). These findings indicate that approximately 15% dietary protein is sufficient for optimal HPG development, suggesting moderate protein supplementation effectively enhances honey bee physiology without requiring excessively high levels. Notably, some commercial artificial pollen diets contain higher protein concentrations that may not confer additional benefits. Ricigliano et al. (2022) reported commercial diets contain protein ranging from 14.7–22.0%, with moderate concentrations closer to our effective formulations. Survival analysis revealed that honey bees fed MP, CB-Ch, CB-Po, and CB-Bu diets exhibited longer lifespans and higher survival rates compared to Sr controls and CB-Co treatments, with CB-Po showing the most pronounced survival benefits (Fig. 1). Over 75% of individuals fed CB diets survived to day 18, compared to rapid mortality in Sr controls, indicating enhanced early adult survival and population stability. These results demonstrate that chicken meat-blood (CB) diets effectively support honey bee health and longevity, comparable to natural pollen (MP), consistent with recent findings on protein-enhanced artificial diets (Chuttong et al., 2025; Danmek et al., 2025).

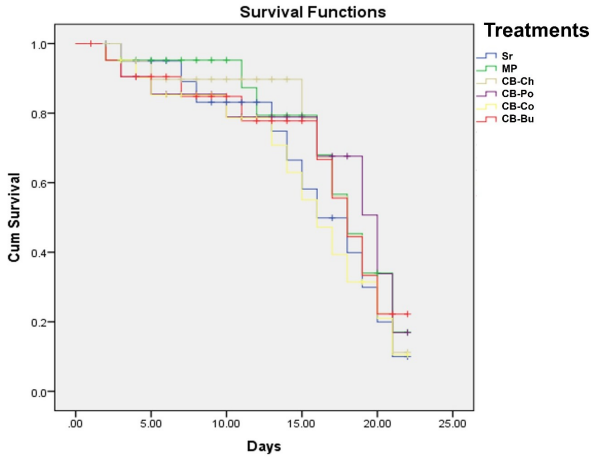

Fig. 1: Kaplan-Meier survival curves of honey bees fed CB diets and controls over time; MP = mixed pollen diet, CB-Ch = CB + chicken blood, CB-Po = CB + pork blood; CB-Co = CB + cow blood, CB-Bu = CB + buffalo blood.

Gut Microbiome

The addition of feed (pollen) enhances both richness and evenness, while also diversifying phylum-level composition, consistent with previous observations that pollen serves as a critical resource supporting the honey bee microbiome (Anderson et al., 2013). At the genus level (Fig. 2), the gut microbiota of honey bees is dominated by five core bacterial genera: *Lactobacillus*, *Bombilactobacillus*, *Gilliamella*, *Snodgrassella*, and *Bifidobacterium*. In addition, three non-core genera of *Frischella*, *Bartonella*, and *Commensalibacter* are commonly detected. Furthermore, several environmentally associated genera, including Parasaccharibacter, Saccharibacter, and Apilactobacillus, are also present in the worker honey bee gut community (Luo et al., 2024; Zumkhawala-Cook et al., 2024). In this study, the hindgut microbiota of Sr was dominated by *Lactobacillus* together with other core honey bee taxa such as *Snodgrassella*, *Gilliamella*, *Frischella*, and *Bartonella* (consistent with the well-described core honey bee gut microbiome (Raymann & Moran, 2018). *Lactobacillus* is a key beneficial bacterium in the honey bee gut, consistently present across populations. It helps maintain a balanced microbial community, supports nutrient digestion, and inhibits harmful microbes, thereby contributing to overall honey bee health and gut stability (Engel et al., 2012; Kešnerová et al., 2017; Tola et al., 2020). These communities were relatively simple and enriched with Bacillus, reflecting the limited substrate diversity of a Sr group. However, MP shifted the composition by expanding the relative abundance of *Gilliamella* and *Snodgrassella*, both of which are known to participate in complex carbohydrate degradation. This underscores the role of pollen as a key dietary factor that enriches specialized gut symbionts involved in polysaccharide fermentation and nutrient provisioning to the host (Ricigliano et al., 2017). Consistent with this, previous studies have shown that pollen consumption by honey bees increases the abundance of both total and core bacterial communities in the hindgut (Luo et al., 2024). As shown in Fig. 2, the relative abundance of bacterial genera in the hindguts of honey bees remained largely consistent across different dietary treatments, including MP and all CB diets, as well as the Sr group. Stacked bar plots illustrate that the composition of the core and non-core gut microbiota were maintained across all treatments, with only minor variations observed in environmentally and pathogen associated taxa. These results indicate that core genera

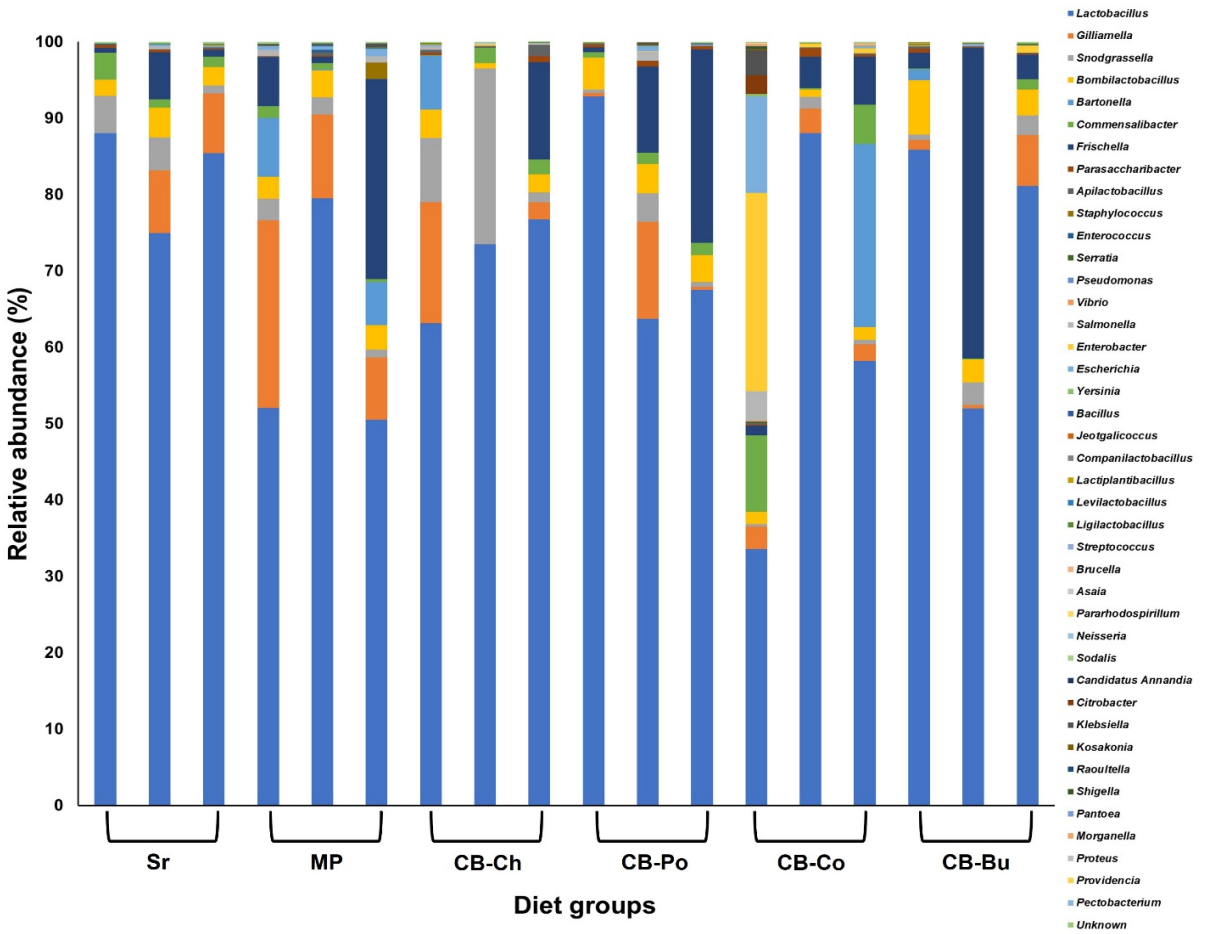

Fig. 2: Relative abundance of bacterial genera in honey bee hindguts under different dietary treatments. Stacked bar plots show the composition of the hindgut microbiota from worker bees fed with chicken-blood diet and controls. Sr = Syrup without diet, MP = mixed pollen diet, CB-Ch = CB + chicken blood, CB-Po = CB + pork blood, CB-Co = CB + cow blood, CB-Bu = CB + buffalo blood.

have been previously associated with beneficial gut microbiota in other species, suggesting that they might play complementary roles in maintaining the gut ecological balance, enhancing digestion, and supporting the immune system (Olofsson et al., 2023). The findings further underscore the robustness of the honey bee gut microbiome, suggesting that even under altered dietary conditions, the core microbial community is preserved, ensuring functional continuity of the gut ecosystem (Raymann & Moran, 2018; Tola et al., 2020; Luo et al., 2024).

The species-level analysis revealed that all experimental diets were primarily dominated by *Lactobacillus* spp., with *Lactobacillus* sp. IBH004 and *L. kullabergensis* being particularly abundant. In addition, the consistent presence of *Snodgrassella alvi* and *Gilliamella apicola* was observed across groups (Fig. 3). Interestingly, these results similar to several previous reports on the honey bee gut microbiome, which typically identified *Lactobacillus* as the dominant bacterial taxa (Luo et al., 2024; Zumkhawala-Cook et al., 2024). The absence or very low detection of environmental and pathogenic bacteria in our study might be explained by differences in diet, experimental conditions, or geographic and seasonal factors that shape the microbial community structure. The findings of this study highlight that honey bees receiving CB diets exhibited increased levels of beneficial gut bacteria and decreased levels of pathogenic bacteria same as MP (Castelli et al., 2022). In some instances, feeding honey bees with artificial pollen diets can alter gut microbial composition, reduce microbial diversity, and decrease the abundance of potentially beneficial bacteria, thereby increasing susceptibility to pathogens (Powell et al., 2023). However, exclusive feeding with Sr has been shown to diminish the diversity of gut bacterial communities in honey bees. Accordingly, the provision of nutritionally balanced diets is critical for promoting and maintaining a diverse and functionally robust gut microbiome (Geldert et al., 2021).

These findings underscore the critical role of high-quality nutrition in maintaining honey bee health**,** consistent with previous reports indicating that consumption of fresh pollen has long been recognized for supporting robust gut microbiota and overall well-being (Luo et al., 2024). In the present study, diets formulated with chicken meat as the primary protein source and supplemented with livestock blood (CB diets) promoted higher abundances of beneficial gut bacteria while reducing the prevalence of pathogenic taxa, yielding effects comparable to those observed in pollen-fed bees (MP). These findings indicate that the experimentally

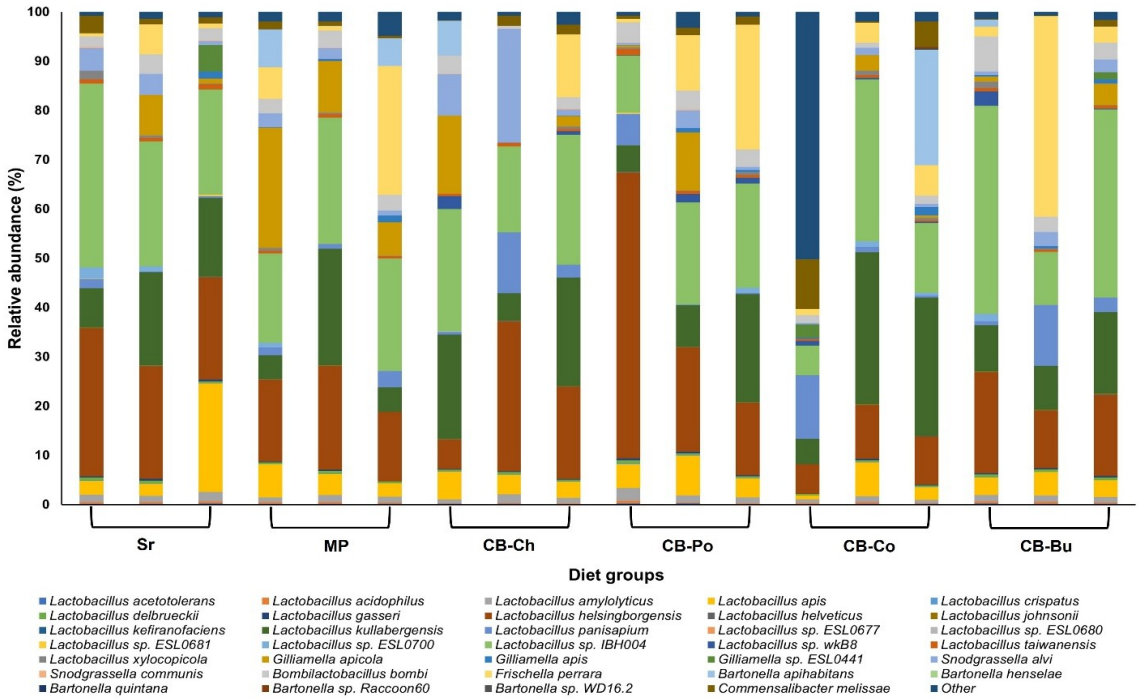

Fig. 3: Relative abundance of bacterial species in honey bee hindguts under different dietary treatments. Stacked bar plots show the composition of the hindgut microbiota from worker bees fed with chicken-blood diet and controls. Sr = Syrup without diet, MP = mixed pollen diet, CB-Ch = CB + chicken blood, CB-Po = CB + pork blood, CB-Co = CB + cow blood, CB-Bu = CB + buffalo blood.

formulated artificial pollen diets, particularly the CB-Po and CB-Co formulations, can effectively mimic the nutritional benefits of natural pollen (MP) by promoting gut microbial balance and enhancing host resilience. Although this study was conducted under controlled laboratory conditions, which may constrain the generalization of our findings to field settings, the results provide valuable insights into honey bee gut microbiota and dietary effects, particularly in the context of artificial pollen diets formulated with animal-derived ingredients.

Conclusion

Natural pollen remains the ideal protein source for honey bee nutrition, but alternatives are critical during dearth periods. This study demonstrates that chicken meat-based diets supplemented with livestock blood match natural pollen in supporting HPG acini development, lifespan extension, and beneficial gut microbiota profiles while reducing pathogens. These cost-effective formulations leverage locally abundant protein sources, offering Thai beekeepers a practical solution for colony maintenance during pollen scarcity. Although conducted under controlled laboratory conditions, these diets warrant field trials to validate their effectiveness and practicality under natural colony conditions.

DECLARATIONS

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Conflict of Interest: The authors state that there are no conflicts of interest with this study.

Data Availability: All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Ethics Statement: Ethics approval for the experiment was obtained through the Laboratory Animal Research Center, University of Phayao under approval number 1-015-68.

Author's Contribution: Sutthisak Yarungsee: Investigation, Methodology, Formal analysis, Data curation, Writing-original draft. Pornprapa Sanluang: Investigation, Methodology, Formal analysis, Data curation, Writing-original draft. Tippapha Pisithkul: Methodology, Formal analysis, Data curation, Writing-original draft. Chuleui Jung: Conceptualization, Supervision, Writing-review & editing, Funding acquisition. Bajaree Chuttong: Conceptualization, Supervision, Formal analysis, Investigation, Writing-review & editing, Funding acquisition. Khanchai Danmek:

Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing-original draft, Writing-review & editing, Funding acquisition.

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REFERENCES

Anderson, K.E., Sheehan, T.H., Mott, B.M., Maes, P., Snyder, L., Schwan, M.R., Walton, A., Jones, B.M., & Corby-Harris, V. (2013). Microbial ecology of the hive and pollination landscape: Bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (*Apis mellifera*). *PLoS One*, 8(6), e83125. https://doi.org/10.1371/journal.pone.00

Ansaloni, L.S., Kristl, J., Domingues, C.E., & Gregorc, A. (2025). An overview of the nutritional requirements of honey bees (*Apis mellifera* Linnaeus, 1758). *Insects*, 16(1), 97. https://doi.org/10.3390/insects16010097

Braglia, C., Rudelli, C., Tinti, A., Bocquet, M., Isani, G., Bulet, P., Giacomelli, A., Di Gioia, D., & Alberoni, D. (2025). Unravelling pollen diet and microbiome influence on honey bee health. *Scientific Reports*, 15(1), 13474. https://doi.org/10.1038/s41598-025-96649-5

Bryś, M.S., Skowronek, P. and Strachecka, A. (2021). Pollen diet—properties and impact on a bee colony. *Insects*, 12(9), 798. https://doi.org/10.3390/insects12090798

Castelli, L., Branchiccela, B., Romero, H., Zunino, P., & Antúnez, K. (2022). Seasonal dynamics of the honey bee gut microbiota in colonies under subtropical climate. *Microbial Ecology*, 83, 492-500. https://doi.org/10.1007/s00248-021-01756-1

Chuttong, B., Praphawilai, P., Ghosh, S., Jung, C., Jang, H., Sun, S., Wu, M.C., & Danmek, K. (2025). Evaluating the potential of a hydrolyzed chicken-based diet as a supplementary feed for enhancing honey bee health, lifespan, and gut microbiome. *Journal of Applied Biotechnology Reports*, 12(3), 1723-1735. https://doi.org/10.30491/jabr.2025.504841.1844

Danmek, K., Hongsibsong, S., Klaithin, K., Klaitanoad, S., Auearchin, T., Wu, M.C., Jung, C., & Chuttong, B. (2025). Enhancing the health and lifespan of Honey Bees (*Apis mellifera* L.) with an innovative soybean-based diet supplemented with porcine blood. *Journal of Entomological Science*, 60(3), 411-423. https://doi.org/10.18474/JES24-49

Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L.P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J.L. & Alaux, C. (2013). Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter?. *PloS One*, 8(8), e72016. https://doi.org/10.1371/journal.pone.0072016

Duarte, R.T., Carvalho Simões, M.C. & Sgarbieri, V.C. (1999). Bovine blood components: fractionation, composition, and nutritive value. *Journal of Agricultural and Food Chemistry*, 47(1), 231–236. https://doi.org/10.1021/jf9806255

Engel, P., Martinson, V.G., & Moran, N.A. (2012). Functional diversity within the simple gut microbiota of the honey bee. *Proceedings of the National Academy of Sciences*, 109(27), 11002-11007. https://doi.org/10.1073/pnas.1202970109

Geldert, C., Abdo, Z., Stewart, J., & & Arathi, H.S. (2021). Dietary supplementation with phytochemicals improves diversity and abundance of honey bee gut microbiota. *Journal of Applied Microbiology*, 130(5), 1705-1720. https://doi.org/10.1111/jam.14897

Haydak, M.H., & Tanquary, M.C. (1943). Pollen and pollen substitutes in the nutrition of the honeybee (Technical Bulletin No. 160). *University of Minnesota, Agricultural Experiment Station*, 26.

Jang, H., Ghosh, S., Sun, S., Cheon, K. J., Mohamadzade Namin, S., & Jung, C. (2022). Chlorella-supplemented diet improves the health of honey bee (Apis mellifera). *Frontiers in Ecology and Evolution*, 10, 922741. https://doi.org/10.3389/fevo.2022.922741

Kešnerová, L., Mars, R.A., Ellegaard, K.M., Troilo, M., Sauer, U. & Engel, P. (2017). Disentangling metabolic functions of bacteria in the honey bee gut. *PLoS Biology*, *15*(12), e2003467. https://doi.org/10.1371/journal.pbio.2003467

Korver, D. R. (2023). Current challenges in poultry nutrition, health, and welfare. *Animal*, 17, 100755. https://doi.org/10.1016/j.animal.2023.100755

Lamontagne-Drolet, M., Samson-Robert, O., Giovenazzo, P., & Fournier, V. (2019). The impacts of two protein supplements on commercial honey bee (*Apis mellifera* L.) colonies. *Journal of Apicultural Research*, 58(5), 800-813. https://doi.org/10.1080/00218839.2019.1644938

Latimer, G.W. (2016). Official methods of analysis of AOAC International (20th ed.). Rockville, MD: AOAC International.

Luo, S., Zhang, X., & Zhou, X. (2024). Temporospatial dynamics and host specificity of honeybee gut bacteria. *Cell Reports*, 43. https://doi.org/10.1016/j.celrep.2024.114408

Manning, R. (2016). Artificial feeding of honeybees based on an understanding of nutritional principles. *Animal Production Science*, 58(4), 689-703. https://doi.org/10.1071/AN15814

Mohamed, F. E. R., Mohanny, K., & Mohamed, G. S. (2023). Artificial feeding of honey bee colonies by adding nutritional supplements to pollen substitutes and its effect on the development of the hypopharyngeal gland stages of honeybee workers Apis mellifera L. *SVU-International Journal of Agricultural Sciences*, *5*(2), 29-41. https://doi.org/10.21608/svuijas.2023.211972.1286

Mortensen, A.N., Jack, C.J., Bustamante, T.A., Schmehl, D.R., & Ellis, J.D. (2019). Effects of supplemental pollen feeding on honey bee (Hymenoptera: Apidae) colony strength and Nosema spp. infection. *Journal of Economic Entomology*, 112(1), 60-66.

Noordyke, E.R., & Ellis, J.D. (2021). Reviewing the efficacy of pollen substitutes as a management tool for improving the health and productivity of western honey bee (*Apis mellifera*) colonies. *Frontiers in Sustainable Food Systems*, 5, 772897. https://doi.org/10.3389/fsufs.2021.772897

Olofsson, T.C., Modesto, M., Pascarelli, S., Scarafile, D., Mattarelli, P., & Vasquez, A. (2023). *Bifidobacterium mellis* sp. nov., isolated from the honey stomach of the honey bee *Apis mellifera*. *International Journal of Systematic and Evolutionary Microbiology*, 73, 005766. https://doi.org/10.1099/ijsem.0.005766

Paray, B.A., Kumari, I., Hajam, Y.A., Sharma, B., Kumar, R., Albeshr, M.F., Farah, M.A., & Khan, J.M. (2021). Honeybee nutrition and pollen substitutes: A review. *Saudi Journal of Biological Sciences*, 28(1), 1167-1176. https://doi.org/10.1016/j.sjbs.2020.11.053

Powell, J.E., Lau, P., Rangel, J., Arnott, R., De Jong, T., & Moran, N.A. (2023). The microbiome and gene expression of honey bee workers are affected by a diet containing pollen substitutes. *PLoS One*, 18(3), e0286070. https://doi.org/10.1371/journal.pone.0286070

Raymann, K., & Moran, N.A. (2018). The role of the gut microbiome in health and disease of adult honey bee workers. *Current Opinion in Insect Science*, 26, 97-104. https://doi.org/10.1016/j.cois.2018.02.012

Ricigliano, V.A., Fitz, W., Copeland, D.C., Mott, B.M., Maes, P., Floyd, A.S., Dockstader, A., & Anderson, K.E. (2017). The impact of pollen consumption on honey bee (*Apis mellifera*) digestive physiology and carbohydrate metabolism. *Archives of Insect Biochemistry and Physiology*, 96(3), e21406. https://doi.org/10.1002/arch.21406

Ricigliano, V.A., Williams, S.T., & Oliver, R. (2022). Effects of different artificial diets on commercial honey bee colony performance, health biomarkers, and gut microbiota. *BMC Veterinary Research*, 18(1), 52. https://doi.org/10.1186/s12917-022-03151-5

Tola, Y.H., Waweru, J.W., Hurst, G.D.D., Slippers, B., & Paredes, J.C. (2020). Characterization of the Kenyan honey bee (*Apis mellifera*) gut microbiota: A first look at tropical and Sub-Saharan African bee-associated microbiomes. *Microorganisms*, 8(11), 1721. https://doi.org/10.3390/microorganisms8111721

Zhang, J., Zou, Y., Yan, B., Zhang, N., Zhao, J., Zhang, H., Chen, W., & Fan, D. (2023). Microwave treatment on structure and digestibility characteristics of Spirulina platensis protein. *Current Research in Food Science*, 7, 100581. https://doi.org/10.1016/j.crfs.2023.100581

Zumkhawala-Cook, A., Gallagher, P., & Raymann, K. (2024). Diet affects reproductive development and microbiota composition in honey bees. *Animal Microbiome*, 6, 64. https://doi.org/10.1186/s42523-024-00350-3