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RESEARCH

The potential of mulberry leaf protein concentrate as a supplementary feed on the health and lifespan of honey bees (*Apis mellifera* L.)

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Background: In situations where natural pollen sources are insufficient, it becomes crucial to explore alternative proteins for honey bees. Utilizing mulberry leaf protein concentrate (LPC) emerges as a vital strategy to enhance the nutritional quality of dietary supplements for honey bees, thus mitigating the effects of limited pollen availability. Moreover, it acts as a dietary supplement that positively influences honey bee longevity and health. This study aims to evaluate the effects of incorporating mulberry LPC into honey bee nutritional supplements on maintaining bee health and prolonging their lifespan.
Results: Mulberry LPC exhibited high protein content (28.60% ± 3.22%), dry matter (86.55% ± 4.56%), and low levels of fiber (3.16% ± 0.25%), ether extract (3.12% ± 0.25%), and ash (0.71% ± 0.10%), respectively. The essential amino acid composition derived from mulberry LPC revealed elevated values of leucine and lysine, which are necessary for honey bee development. Through a comprehensive investigation of mulberry LPC supplementation in pollen patty on honey bee physiology and life span, the treatment administering 2.5%–5.0% mulberry displayed a significant increase ($p < 0.05$) in acini sizes and prolonged the life span when compared to the control group fed solely with sucrose syrup.
Conclusions: This finding is the first report highlighting the potential of mulberry LPC as a novel supplement feed for honey bees. Mulberry LPC demonstrated notable characteristics, including a high protein content. Furthermore, the essential amino acid composition of mulberry LPC showed elevated levels of leucine and lysine. Our results signify the beneficial impact of mulberry LPC in honey bee nutrition, suggesting its potential as a viable dietary intervention to improve honey bee health and life span. Further research in this domain holds promise for advancing beekeeping practices and ensuring the sustainability of honey bee populations.

Keywords: bee health, honey bee nutrition, life span, mulberry leaf protein concentrate, supplement

Introduction

The honey bees are the primary pollinators globally, responsible for approximately 90% of the world's food production (Feketéné Ferenczi et al. 2023). According to Klein et al. (2007), more than 30% of crops directly rely on pollinators. Furthermore, Patrício-Roberto and Campos (2014) highlight that 84% of cultivated plant species depend on insects for pollination, with western honey bees standing out as the most economically valuable pollinators worldwide. However, recent shifts in climatic conditions have posed significant challenges to ecosystems, impacting the intricate relationships between flowers and pollinators. Changes in climate patterns can alter plant phenology and flowering schedules, presenting nutritional challenges for insects' reliance on protein-rich flower pollen (Abrol 2012).

Additionally, the severe weather condition in certain seasons limits honey bee foraging opportunities, reducing their ability to gather resources. Consequently, supplemental diets such as pollen or pollen substitutes become critical for providing essential nutrients to bee colonies, facilitating brood rearing, population growth, and survival during severe seasons (Dastouri et al. 2007; Goodwin et al. 1994).

Pollen patty is commonly used to provide the necessary protein source for honey bees when there is a shortage of food resources in the environment. This is typically offered in the form of a patty, which can be placed inside the hive and help ensure that the bees have enough feed to survive and thrive (Jang et al. 2022). In addition, pollen patties supplemented with protein sources can help to strengthen the immune systems of honey bees, making them more resistant to varroa mites and other pests and diseases (Annoscia et al. 2017; DeGrandi-Hoffman et al. 2020). When pollen cannot be found in nature, numerous commercial products available on the market are designed to enhance and sustain bee colonies. These products contain plant-based proteins other than soy sources, such as MegaBee®, FeedBee®, and Ultra Bee®. This implies that plant-based protein sources could serve as substitutes for honey bees, exhibiting effects similar to those of natural pollen (Lamontagne-Drolet et al. 2019).

During times of food scarcity in their environment, pollen patties serve as crucial sources of essential protein for honey bees. Pollen patties supplemented with 2% *Chlorella* as a protein source can be placed inside the hive, ensuring that the bees receive sufficient nutrition to survive (Jang et al. 2022). Supplementing pollen to the honey bee diet can reduce the negative effects of Varroa mite infestation and associated viral infections, while also maintaining low virus levels and enhancing survival. This is attributed to the beneficial impact of pollen on immunity and colony growth (Annoscia et al. 2017; DeGrandi-Hoffman et al. 2020). In situations where natural pollen is scarce, numerous commercial products available on the market are formulated to fortify and maintain bee colonies. These products incorporate plant-based proteins, such as MegaBee®, FeedBee®, and Ultra Bee®, as substitutes for soy-based sources. This suggests that plant-based protein sources have the potential to emulate the effects of natural pollen, thereby serving as viable alternatives for honey bees (Lamontagne-Drolet et al. 2019).

In recent years, there has been increasing interest and numerous reports concerning the utilization of proteins derived from plant leaves. Several studies have highlighted the nutritional value and functional properties of proteins extracted from plant leaves, indicating their potential as viable alternatives to conventional protein sources. For instance, a study conducted by Santamaría-Fernández and Lübeck (2020) examines the nutritional composition of proteins extracted from various plant leaves, emphasizing their rich amino acid profile and potential health-promoting properties.

Mulberry (*Morus alba* L.) is an economically important tree, providing many valuable inputs for food and feed. The most common species of mulberry can yield a leaf biomass of approximately 25 to 30 tons/ha/year with a shorter harvesting interval of about 9 to 10 weeks (Hassan et al. 2020). In Thailand, mulberry leaf production consists of 74,182 growers and 21,780.90 acres of cultivation area, while mulberry fruit cultivations have 901 growers and 441.30 acres of cultivation area (Choosung et al. 2022). Mulberry leaves are rich in nutritional values of protein (14%–34%), carbohydrates (9%–39%), metabolizable energy (1,130–2,240 kcal/kg), high in vivo dry matter (DM) digestibility (75%–85%), and absence of anti-nutritional factors (Hassan et al. 2020; Kandylis et al. 2009). Plant leaves are indeed cost-effective. However, lignocellulose, a primary constituent of secondary plant cell walls, exhibits high resistance to degradation compared to primary cell walls, posing challenges for many animals in utilizing it as a food source (Tokuda 2019). Unlike ruminants, honey bees cannot directly metabolize plant cell walls. Consequently, the potential utilization of mulberry leaf protein by honey bees might be achieved by producing leaf protein concentrate (LPC).

Numerous varieties of mulberry plants worldwide contain a substantial amount and quality of leaf protein fraction, proximately comprising 11%–25% of protein content. The protein fractions of LPC can be prepared using standard methods, including chemical extraction, enzyme-assisted extraction, and heat coagulation, among others, which separate the proteins based on their solubility in different systems (Kandylis et al. 2009; Kaur and Bhatia 2022). Therefore, mulberry LPC is presumed to be suitable as supplementary feed for honey bees and to play a valuable role in global agriculture. However, the supplementation of LPC in honey bee feed necessitates a comprehensive understanding of LPC properties, including microstructure, nutritive values, mineral contents, and benefits as a high-quality supplement or replacement for pollen-based patties in honey bee feeding. Furthermore, an examination of the physicochemical and functional properties of mulberry LPC is crucial, particularly concerning the health and growth of honey bees. This knowledge gap arises from the current challenges faced by honey bees in pollen-deficient areas, and the need for a sustainable solution prompts an investigation into utilizing the nutritional potential of abundant and protein-rich mulberry LPC. Thus, the objective of the present study was to evaluate the nutritional value of mulberry LPC, partially substituting pollen patty and its effect on the health and lifespan of honey bees.

Materials and Methods

Mulberry leaf samples

The mulberry leaves were harvested from the plantation at the Department of Entomology and Plant Pathology, Faculty of Agriculture, Chaing Mai University, Thailand (N 18.7942141 and E 98.9612368) in November 2023. The stalks were removed from the leaves, then washed and stored in airtight containers at room temperature (30°C) for further experimentation.

Preparation of mulberry leaf protein concentrate

The methodology utilized in this study was adapted from the approach developed by Kaur and Bhatia (2022). One kg of fresh mulberry leaves was submerged in deionized water, maintaining a solid-liquid ratio of 1:3, and the mixture was stirred to achieve a homogeneous slurry. Subsequently, the pH of the homogenized slurry was adjusted to 12 by adding 1N NaOH to maintain alkaline conditions at room temperature for 3 hours. Filtration through cotton fabric separated the green juice supernatant, which mostly contained the soluble protein fraction. The pH of the supernatant was then adjusted to 4 using 1N HCl, and subsequently, it was heated in a water bath at 80°C–90°C for 10 minutes to induce coagulation of the leaf protein. Overnight sedimentation at room temperature facilitated the separation of the LPC fraction from the supernatant. To eliminate residual fiber from the LPC cellulose was added at a concentration of 1% (v/v) (commercial cellulase iKnowZymeTM®, purchased from Rechbiotechnology Co., Ltd., Pathum Thani, Thailand), and the sample was incubated at 60°C for 3 hours. The resulting LPC precipitates were washed and liquefied using deionized water and then powdered using the freeze-drying process. The final product, referred to as mulberry LPC power, was preserved in polyethylene bottles and stored in the refrigerator at 4°C for further experiments (Fig. 1).

Microscopic characterization and nutritional composition analyses of mulberry leaves and mulberry leaf protein concentrate

The microstructures of mulberry leaves and LPC were analyzed by scanning electron microscopy (SEM). The samples underwent sputter-coating with gold and were examined using SEM (Prisma E; Thermo Fisher Scientific, Waltham, MA, USA).

All chemical analyses were conducted in triplicate. Samples were ground through a 1.0 mm mesh and analyzed for DM, crude protein, crude fat, crude fiber, and ash which were determined according to the Association of Official Analytical Chemists (AOAC) procedures (Latimer 2016). The amino acid content analysis followed the methodology of Ghosh and Jung (2022) using Sykam Amino Acid Analyzer S433 (Sykam GmbH, Bayern, Germany) with a Sykam LCA L-07 column, following the standard method of AOAC. To prepare the samples, hydrolysis was performed in 6 N HCl for 24 hours at 110°C under a nitrogen atmosphere, followed by concentration in a rotary evaporator. The concentrated samples were reconstituted with a sample dilution buffer provided by the manufacturer (physiological buffer, 0.12 N citrate buffer, pH 2.20), and the hydrolyzed samples were then analyzed for amino acid composition.

The determination of the total polyphenol content (TPC) of mulberry LPC was carried out with modifications based on the method for Indian herbal teas (Naithani et al. 2006). The TPC was expressed as gallic acid equivalents (GAE) in milligrams per gram of the sample. The total flavonoid content (TFC) in the sample was determined using the aluminum nitrate (Al $(NO_3)_3$) colorimetric method, with

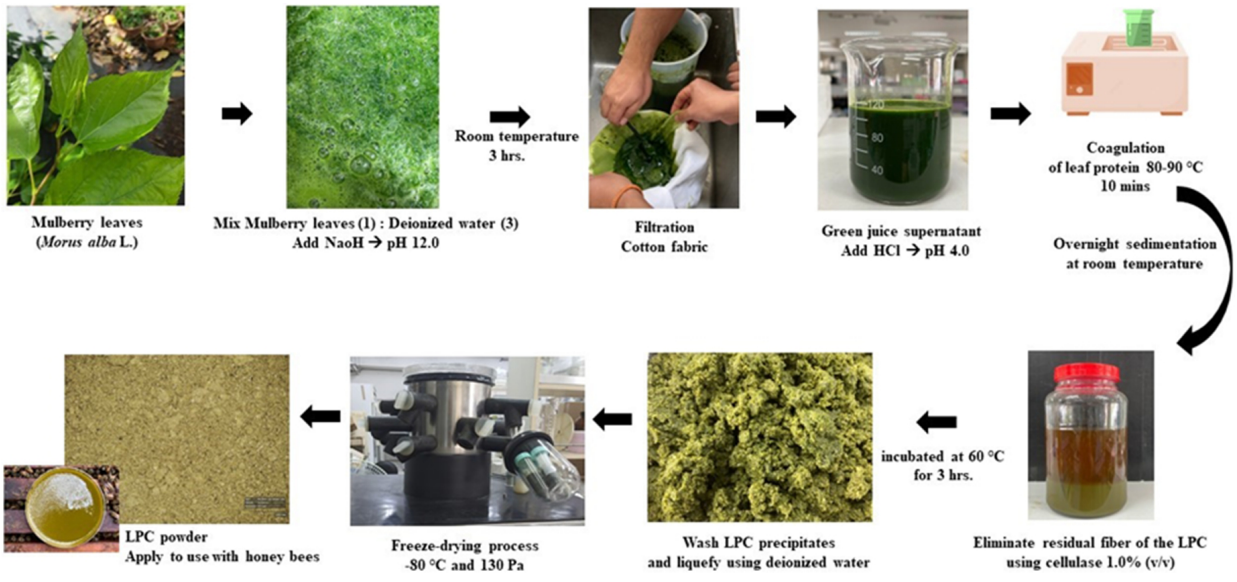


Fig. 1 Extraction process of leaf protein concentrate (LPC) from mulberry leaves for bee supplement production.

modifications from the method of Wu et al. (2022). The total flavonoid content was expressed as quercetin equivalents (QE) in milligrams per gram of the sample. A standard curve of QE was prepared at 0–100 μg/mL, which was used to calculate the TFC of mulberry LPC.

Preparation of honey bee colonies

Healthy *A. mellifera* colonies were maintained in the apiary at Chiang Mai University, Thailand. These colonies of honey bees were meticulously managed and adhered to standard beekeeping guidelines. For this study, sealed brood frames were removed from the bee colony and transferred to an insect growth chamber (temperature: 34°C ± 1°C, relative humidity: 60% ± 10%, and darkness) until the pupae developed into adult bees, following the protocol outlined by Hsu et al. (2021).

Evaluation of a supplement feed containing mulberry leaf protein concentrate

The experiment consisted of four groups, each comprising 30 newly emerged adult honey bees. These bees were housed in transparent acrylic cages measuring 15 cm × 10 cm × 15 cm. Air circulation was facilitated through the lid of the experimental cage using a nylon grid with a mesh size of 5 mm or less. All cages were maintained in the growth chamber, and each was supplied with 20 mL of 50% (w/w) sucrose solution in the syrup feeder.

To assess the effect of mulberry LPC as a supplemental feed on the health and lifespan of honey bees, four treatments of butterfly needle pollen (*Bidens pilosa* L.) were prepared. These treatments involved replacing 0%, 2.5%, 5.0%, and 10.0% (w/w) of LPC with pollen. Each pollen patty was mixed with 50% (w/w) sucrose solution at a mass ratio of 2:1, and then 2.0 g of the resulting patty was placed in a plastic petri dish within the cage.

Throughout the experiment, each cage was replicated three times in a dark incubator maintained at a temperature of 34°C ± 1°C and relative humidity of 60% ± 10% (Hsu et al. 2021). Patty and sucrose solutions were replaced every 3 days. Bee mortality was recorded, and the number of dead bees was counted daily until 21 days after emergence.

Furthermore, the development of hypopharyngeal glands (HPG) and acini was examined in 6-day-old honey bees. The measurement of HPGs was adapted from the protocol described in Corby-Harris and Snyder (2018). Three honey bee samples were selected from each treatment group, and their heads were dissected with stainless steel forceps to expose their HPGs. Subsequently, images were captured under a stereo microscope (Leica Microsystems - Fluorescence stereo microscopes Leica M205 FCA & Leica M205 FA, Wetzlar, Germany) and a 3-dimensional digital microscope camera (HRX-01, RX-100; Hirox, Tokyo, Japan). For each sample, the diameters of 10 randomly selected acini, with clearly focused borders, were measured in pixels and converted to millimeters (three replicates). The averages of the 10 individual acini measured per bee head were used for statistical analysis.

Statistical analysis

A statistical analysis of variance of one-way ANOVA, followed by Duncan's multiple range test, was used to compare the parameters, as the level of significance was $p < 0.05$, employing SPSS version 26.0 (IBM Co., Armonk, NY, USA). Kaplan-Meier survival analysis was used to determine the effect of mulberry LPC supplements on the life span of honey bees. Differences in the time distributions between groups were tested for statistical significance.

Results

SEM characterization of mulberry leaves and mulberry leaf protein concentrate

The mulberry leaves utilized in this study were characterized for their LPC content and found to be a promising source of protein. Employing a traditional technique involving alkali extraction and acid precipitation, protein extraction from plants was carried out. Additionally, employing biological treatment with cellulase significantly enhanced the digestibility of mulberry LPC by eliminating a substantial portion of the fiber. This enzymatic treatment facilitated the breakdown of plant fiber, resulting in reduced particle size and increased specific surface area of proteins. Examination of the mulberry leaf microstructures through SEM revealed a rough surface characterized by sheet structures and numerous dispersed small fragments. In contrast, the protein molecules extracted from mulberry LPC displayed relatively smaller and rougher protein particle shapes, with some small spherical particles distributed across their surface (Fig. 2).

Nutritional composition of mulberry leaves and mulberry leaf protein concentrate

Figure 3 illustrates the nutritional composition of both mulberry leaves and LPC. Mulberry LPC contained notably high protein (28.60% ± 3.22%) and DM (86.55% ± 4.56%), along with low fiber (3.16% ± 0.25%), ether extract (3.12% ± 0.25%), and ash (0.71% ± 0.10%), respectively. In contrast, mulberry leaves contained significantly lower protein content (16.13% ± 1.63%) but high fiber content (14.97% ± 2.61%) compared to mulberry LPC ($p < 0.05$). Moreover, mulberry LPC demonstrated a slight increase ($p \geq 0.05$) in crude fat content (3.12% ± 0.67%) compared to crude fat of mulberry leaves (2.32% ± 0.17%). These findings suggest that the method employed effectively extracted protein from mulberry leaves. Additionally, the study revealed TPC and TFC in mulberry LPC were 26.79 ±

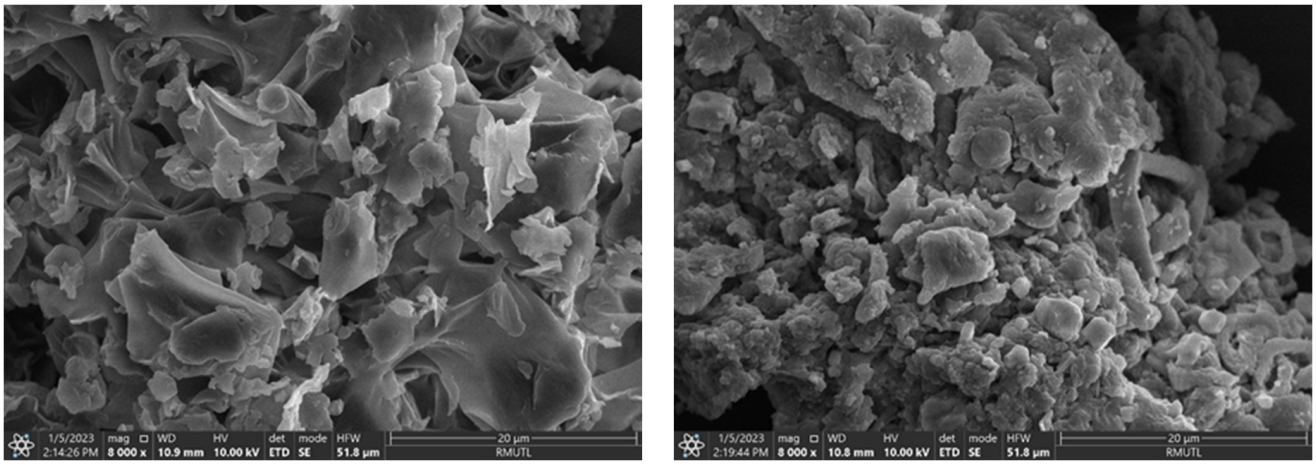


Fig. 2 (A) Scanning electron microscopy images of mulberry fractions, depicting mulberry leaves, (B) and mulberry leaf protein concentrate.

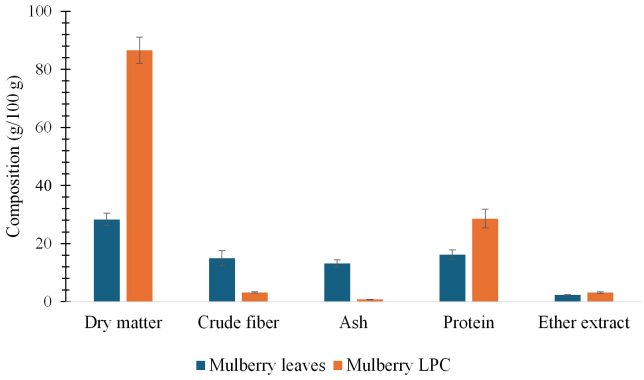


Fig. 3 Nutritive values of mulberry leaves and mulberry leaf protein concentrate (LPC).

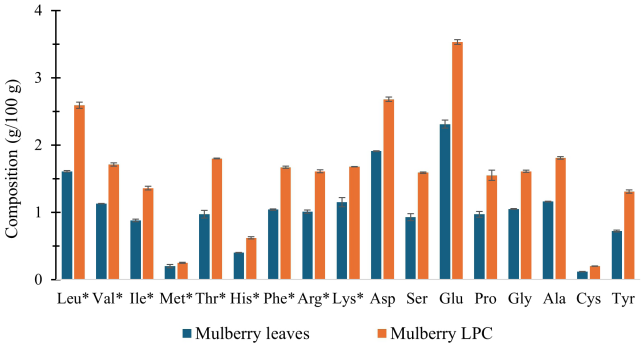


Fig. 4 Amino acid compositions of mulberry leaves and mulberry leaf protein concentrate (LPC). *Essential amino acid for honey bees.

1.83 GAE mg/g and 82.90 ± 13.40 QE mg/g, respectively.

The role of mulberry LPC as a protein supplement for honey bees was the focus of our study, with particular attention given to assessing the amino acid composition. Seventeen amino acids were identified and quantified, excluding tryptophan due to its complete degradation during acid hydrolysis. The amino acid profile of mulberry LPC closely resembled that of the leaves, although LPC contained higher amounts of individual amino acids, as depicted in Figure 4. Except for tryptophan, all essential amino acids (EAAs) vital for honey bees were present in mulberry LPC. Notably, EAAs crucial for honey bee development, such as leucine and lysine, were among the most abundant. Furthermore, non-EAAs, including glutamic acid, aspartic acid, alanine, and proline, exhibited higher values.

Effect of mulberry leaf protein concentrate supplementation on honey bee

The comparison between groups receiving no-protein supplements and those receiving protein supplements throughout the experiment highlighted significant differences in LPC supplementation. Notably, on 6th day, honey bees exclusively fed with syrup without protein supplementation exhibited significantly smaller acini sizes (0.092 ± 0.004 mm) compared to those fed with pollen patty treatments (0.152 ± 0.007 mm) ($p < 0.05$) as illustrated in Figure 5.

Furthermore, the HPG gland acini of treatments fed with pollen patty (0.161 ± 0.007 mm), 2.5% LPC supplement (0.159 ± 0.007 mm), and 5.0% (w/w) LPC supplement (0.156 ± 0.007 mm) were significantly higher ($p < 0.05$) from 10.0% (w/w) LPC supplement (0.143 ± 0.007 mm) and control without pollen (0.106 ± 0.007 mm) respectively (Fig. 6). These findings suggest that honey bees fed with an appropriate level of mulberry LPC (2.5%–5.0%) exhibited significantly larger acini sizes, emphasizing the critical role of protein in honey bee health.

In terms of survival analysis, bees fed with pollen patty and mulberry LPC supplements demonstrated significantly longer survival rates than those fed only with sucrose syrup (Kaplan–Meier survival analysis) (Fig. 7). Notably, using a 50% (w/w) sucrose solution to feed newly emerged bees ensured the lowest survival rate within 13 days from the

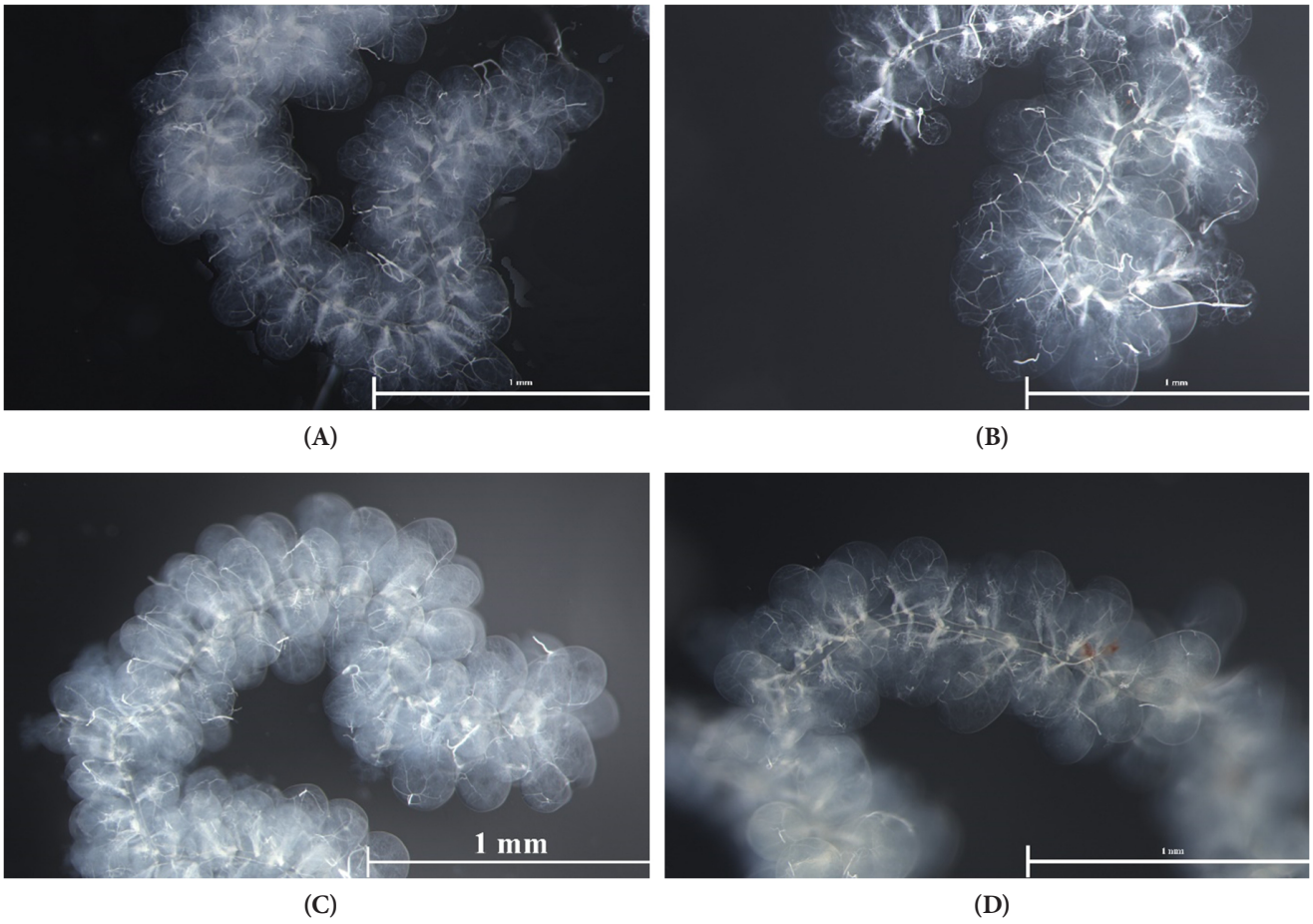


Fig. 5 The development of hypopharyngeal gland acini in honey bees fed with different supplement compositions: no pollen (A), 2.5% leaf protein concentrate (LPC) supplement (B), 5.0% LPC supplement (C), and 10.0% LPC supplement (D).

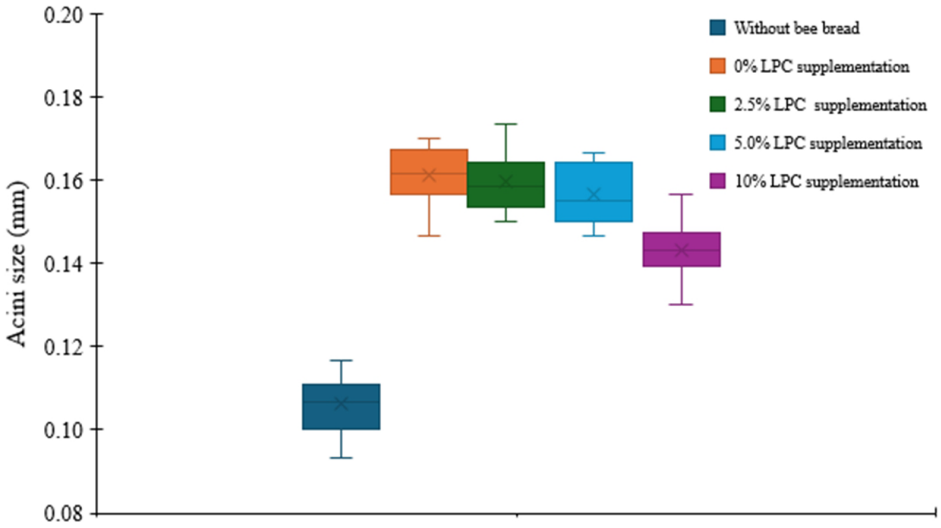


Fig. 6 Acini sizes (mm) of honey bee hypopharyngeal glands measured at day 6, with 10 individual acini measured/bee head ($p < 0.05$). LPC: leaf protein concentrate.

start of the experiment. When compared within four LPC supplements, all showed no significant difference ($p \geq 0.05$). This suggests that feeding the mulberry LPC at a level of 2.5%–5.0% did not have a detrimental impact on the health and lifespan of honey bees.

Discussion

Mulberry LPC, obtained through extraction procedures involving alkali extraction and acid precipitation, exhibited protein particles characterized by irregular, rough shapes reminiscent of the glutelin structure. Generally, proteins are categorized based on their solubility, with albumins

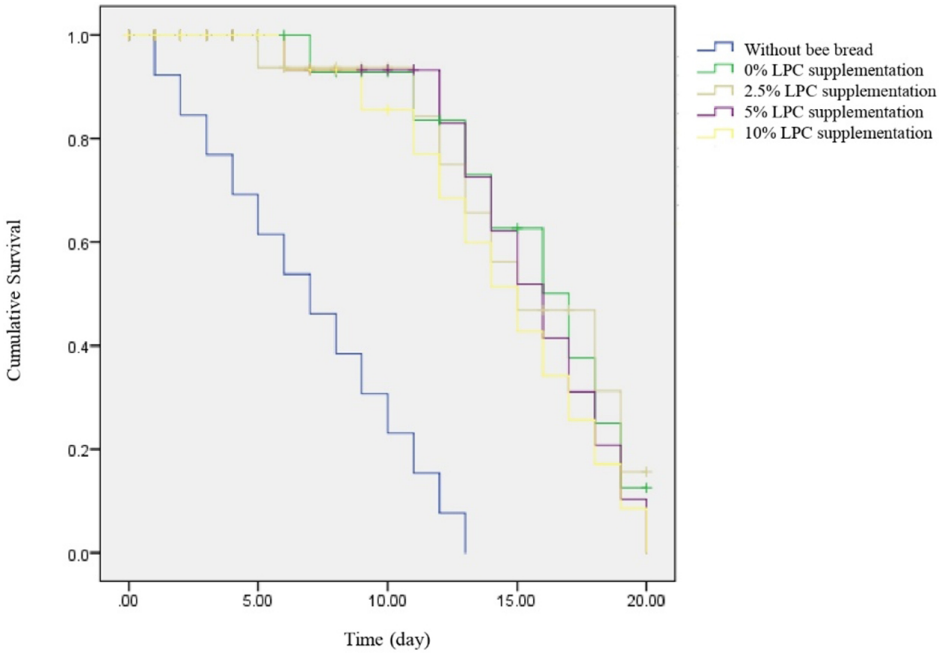


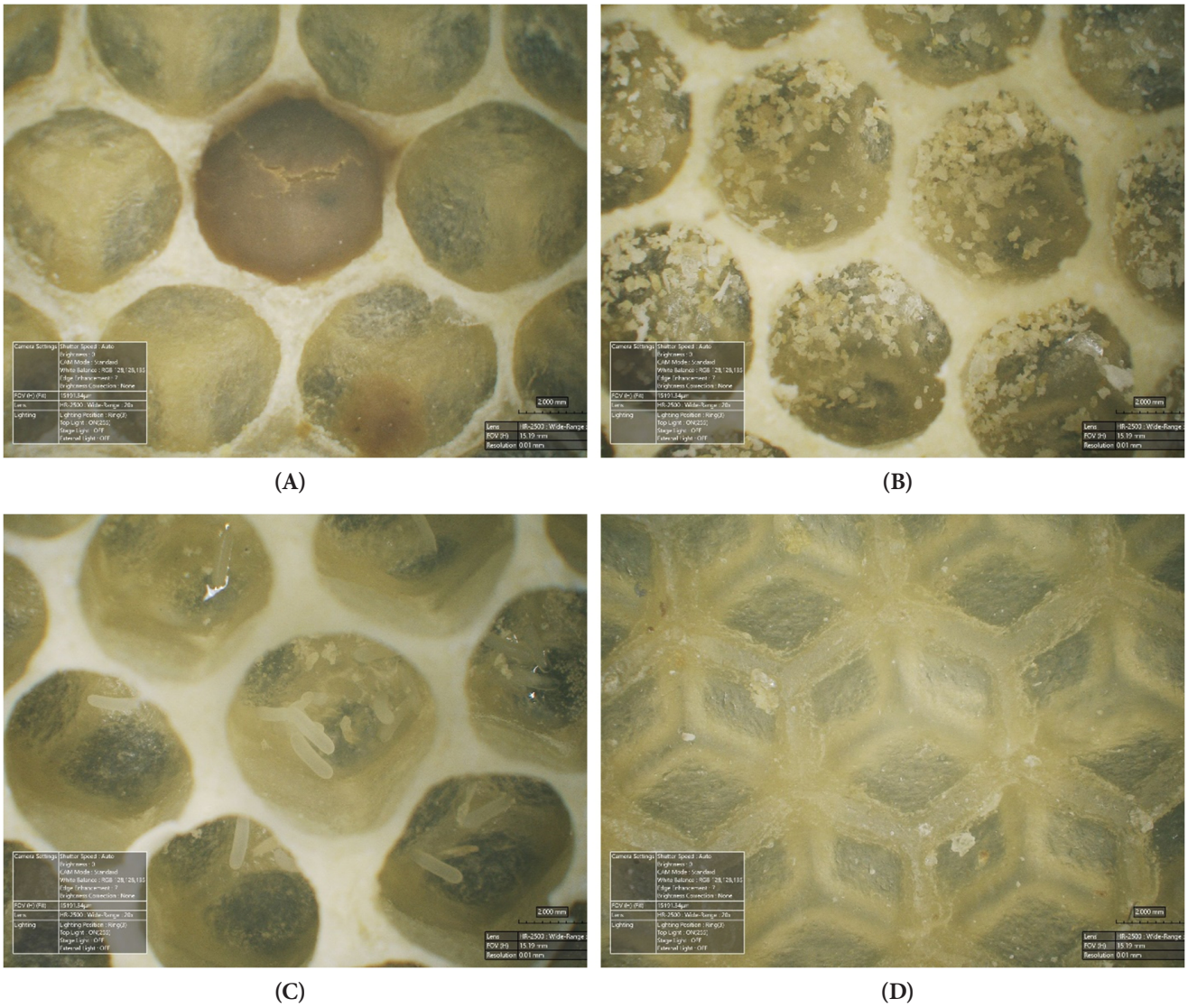
Fig. 7 Survival (day) of honey bee feed on different LPC supplements with 0%, 0.25%, 5.0%, 10% from day one of emergence in the laboratory condition of 25°C and 75% relative humidity. LPC: leaf protein concentrate.

being soluble in aqueous solutions, globulins in salts, glutenin in alkaline solutions, and prolamins in alcoholic solutions (Shewry 2002). According to Kaur and Bhatia (2022) who studied radish LPC, these solutions extracted glutelin (41.49%), prolamins (24.96%), albumins (20.43%), and globulins (13%), respectively. The microstructure of mulberry leaves exhibited a rough surface composed of fiber sheets, whereas mulberry LPC revealed protein particles with small, rough shapes. These observations align with the findings of Sun et al. (2017), who noted that the LPC derived from treated mulberry leaves (*Morus atropurpurea* roxb.) exhibited smaller and more uniform fragments compared to untreated leaves.

This study represents the first discovery suggesting that mulberry LPC could be regarded as a viable protein supplement for honey bees, given its comparable nutritional value to natural feed sources. With a crude protein content of 28%, mulberry LPC was similar to Sun et al. (2017) and surpasses the protein fractions found in other mulberry species (11.75%–23.72%) (Güven 2012; Kandylis et al. 2009). Variations in mulberry species and extraction conditions likely account for these differences. Hence, mulberry LPC, especially 2.5% to 5%, emerges as a potentially beneficial protein supplement capable of enhancing bee health and longevity.

The amino acid profiles of mulberry leaves and LPC exhibit a similar pattern, showing a strong correlation with mulberry protein fractions identified by previous researchers (Sun et al. 2017; Zhang et al. 2014). Honey bees are required to obtain ten EAAs from their diet to facilitate their growth and reproduction (de Groot 1953; Ghosh et al. 2020). Mulberry LPC exhibits higher levels of leucine, lysine, and glutamic acid, consistent with previous studies on bee pollen (Ghosh and Jung 2017; Ghosh et al. 2020).

Similar to other organisms, honey bees necessitate protein for diverse essential physiological functions, which significantly influence their feeding preferences and foraging decisions. Numerous studies have demonstrated that the health of honey bees improves when they consume a well-balanced amount of protein (Brodschneider and Crailsheim 2010; Frias et al. 2016; Tsuruda et al. 2021). The size of the HPG gland and acini in honey bees undergo physiological changes as their role changes with age (Ueno et al. 2015). Acini diameters serve as a well-established measure for assessing the physiological state of the acini and their normal development. In this study, supplementing the pollen diet with 2.5%–5.0% mulberry LPC showed potential for enhancing honey bee health. These findings align with a previous study that showed that giving honey bees protein supplements changed the size of their acini compared to the control group (DeGrandi-Hoffman et al. 2010; Jang et al. 2022; Omar et al. 2017). However, honey bees fed with 10% (w/w) of mulberry LPC exhibited the smallest HPG acini. Similarly, pollen patties containing 10% mulberry led to decreased lifespan in newly emerged workers, indicating suboptimal growth and development conditions. These results are consistent with reports indicating that excessively high protein levels in pollen supplements adversely affect honey bee health and lifespan (Jang et al. 2022). These adverse impacts may be attributed to the elevated levels of phytochemical substances. Additionally, excessively high protein concentrations present in mulberry LPC can also have detrimental effects. In addition, mulberry LPC contains plant secondary metabolites known as plant phytochemicals, which play an important role in defending plants from insect pests and pathogens by acting as poisons and deterrents (Liao et al. 2017). EAAs significantly impact honey bee health by enhancing the size of



(A) **(B)**

(C) **(D)**

Fig. 8 Beeswax is constructed from a foundation sheet, displaying bee bread (A), wax flakes (B), and eggs (C) in caged honey bees. This is concerning their diet of a pollen patty containing 0.0% to 5.0% (w/w) mulberry LPC supplement, compared to the control group without pollen (D).

HPGs, as indicated in the study by Hendriksma et al. (2019), who revealed a substantial difference in head weight between foragers and nurse bees when they received sufficient EAAs. The longevity of honey bees is also influenced by EAAs and the relative ratios of specific macronutrients (Paoli et al. 2014).

Observations of honey bees constructing small combs on foundation sheets, as depicted in Figure 8, during experiments suggest that pollen patties provided all essential nutrients for normal development, supporting a strong correlation between the nutritive values of mulberry LPC and honey bee health and lifespan. As a result, this study offers potential advantages for beekeepers aiming to supplement colonies during periods of pollen shortages or unavailability. Considering the consistent physiological traits of honey bees across diverse geographic regions, the prospective adoption of LPC supplements appears promising for global use and effectiveness.

Conclusions

Our findings suggest that the incorporation of mulberry LPC supplements into pollen patties enhances their efficacy as a protein substitute for honey bees when compared to natural protein sources. Mulberry LPC, ranging from 2.5% to 5.0% (w/w), emerged as a recognized supplementary pollen, positively influencing honey bee health and lifespan. Nonetheless, this research is based on laboratory observations and explains some insight into the impact on worker honey bees. However, the extrapolation of these results to field conditions requires further investigation. Future research should aim to explore the nutritional composition of diverse LPC sources and their applications in honey bee nutrition. This investigation will contribute to supporting the healthy development of colonies. Furthermore, there is a critical need for comprehensive studies to unravel the physiological and biological responses of honey

bees to alternative plant LPC supplements. An explanation of the nutritional profiles of plant LPCs will not only deepen our scientific understanding but also significantly contribute to the economic gains associated with the production of these products.

Abbreviations

DM: Dry matter
LPC: Leaf protein concentrate
SEM: Scanning electron microscopy
AOAC: Association of Official Analytical Chemists
TPC: Total polyphenol content
GAE: Gallic acid equivalents
TFC: Total flavonoid content
QE: Quercetin equivalents
HPG: Hypopharyngeal glands
EAA: Essential amino acid

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Authors' contributions

KD conceptualized the study, developed the methodology, conducted the investigation, prepared the original draft, and acquired funding. MCW contributed to the conceptualization of the study, assisted with validation, and participated in writing, reviewing, and editing. KK and HLN were involved in the investigation. SH contributed to writing, review, and editing. SG assisted with the methodology and participated in writing, review, and editing. CJ was involved in validation, writing, review and editing, and provided supervision. BC contributed to conceptualization, validation, writing the original draft, writing review and editing, and also acquired funding. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

Ethics approval for Experiment No. AG04001/2564 was obtained through the Research Funding Program Management Unit for Human Resources & Institutional Development, Research, and Innovation (PMU-B) of Thailand.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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