Journal of Apiculture
2025.40(4) : 363-373
DOI: 10.17519/apiculture.2025.11.40.4.363

Original research article

Effects of Probiotics on Productivity and Survivorship of European Honeybees (*Apis mellifera*) under Field Conditions in Taiwan

Lekhnath Kafle* and Wen-Hsiung Huang[1]

Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung 912301, Taiwan
[1]International Master Degree Program in Food Science, National Pingtung University of Science and Technology, Pingtung 912301, Taiwan

Abstract The European honeybee (*Apis mellifera*) is well-known for its mild temperament, docility, and higher productivity However, the global population of honeybees has been declining due to various abiotic and biotic factors. Therefore, it is essential to explore strategies for repopulating honeybee populations. This study aimed to enhance the survivorship and productivity of European honeybees by incorporating probiotics into their diets. In this study, we evaluated three commercially available probiotics intended for human use: TS6 Probiotic (TS6), Vitality Probiotic (VP), and Multi-Defense Probiotic (MDP). These were administered at varying doses to evaluate their effects on brood survivorship and hive productivity under field conditions. The results demonstrated that the addition of 1.2 g of TS6 per hive significantly improved brood survivorship among European honeybees. Additionally, hives supplemented with VP at 1.2 g per hive, MDP at 1.2 g per hive, and TS6 at 0.4 g per hive exhibited significantly higher productivity in terms of royal jelly, honey, and pollen compared to other doses. This study found that supplementing probiotics to honeybee's diet positively impacts their survivorship and productivity under field conditions.

Keywords European honeybee, Honey, Pollen, Royal jelly, Probiotic, Survivorship

INTRODUCTION

Bees play a crucial role in the agricultural industry through their bee products and pollination services. Approximately 20,000 bee species exist worldwide (Aljedani, 2022), and only 7-12 species are known to produce honey (Pătruică *et al*., 2013). Among these, the European honeybee (*Apis mellifera*) is particularly notable for its high productivity in terms of bee products, making it a favorite among beekeepers. For generations, humans have relied on bees for various natural products, including honey, pollen, royal jelly, and propolis, all of which hold significant economic value. However, the overall decline in bee health has adversely affected the apiculture industry and reduced the availability of these products. This decline not only impacts the apiculture sector but also has broader environmental consequences (Zapata-Hernández *et al*., 2024).

Probiotics are beneficial bacteria that provide health benefits, such as improved digestion and enhanced immunity, by positively influencing the host's gut microbiota (Evans and Lopez, 2004). While various probiotic products offer distinct health benefits, they all promote growth, development, and immunity in animals, thereby reducing the risk of common diseases. These advantages have led to the increasing popularity of probiotics in various animal industries, including apiculture (Ramos *et al*., 2020; Anee *et al*., 2021; Lika *et al*., 2021). Mojgani *et al*. (2024) also reported an increase in beneficial bacteria within the honeybee gut can enhance bee health by diminishing the prevalence of pathogenic microorganisms.

Among the diverse lactic acid bacteria (LAB) with

http://journal.bee.or.kr/
Received 11 June 2025; Revised 10 September 2025; Accepted 15 September 2025
*Corresponding author. E-mail: kafleln@gmail.com

probiotic potential, strains of *Lactobacillus* and *Bifidobacterium* are particularly beneficial (Tejero-Sariñena *et al*., 2012). Other notable probiotic genera include *Streptococcus*, *Bacillus*, *Saccharomyces*, and *Propionibacterium*. Specific strains of probiotic bacteria perform optimally under certain environmental conditions. For instance, *Saccharomyces cerevisiae* has demonstrated positive effects in ruminants, while *Bacillus* spp., *Lactobacillus* spp., and *Enterococcus* spp. yield better results in poultry and pigs (Bermudez-Brito *et al*., 2012). Studies have shown that LAB can inhibit the growth of *Paenibacillus* larvae, the causative agent of American foulbrood disease in honeybees (Moritz *et al*., 2010). LAB thrive in acidic environments, akin to the honeybee gut, where they can inhibit pathogenic bacteria while simultaneously improving gut health, digestion, immunity, and the production of economically important products (Jasim and Fehan, 2017).

Like other animals, honeybees harbor a diverse array of beneficial bacteria in their gut (de Paula *et al*., 2021). This complex microbial community directly influences their health and productivity. However, the decline in honeybee populations may also be linked to an unstable gut microbiome (Kešnerová *et al*., 2017). To ensure healthy and robust bee populations, it is vital to maintain a balanced gut microbiota rich in beneficial bacteria (Horak *et al*., 2020). Pathogenic diseases caused by fungi, bacteria, and viruses have severely impacted the apiculture industry, prompting beekeepers to seek effective solutions. The use of antibiotics has proven problematic due to the emergence of antibiotic resistance among pathogens and pests of bees. This study aimed to identify a more sustainable approach to enhance honeybee strength and productivity through supplementing probiotics in their diets.

Bee colonies face stress from factors such as pesticide exposure, habitat loss, and transportation for pollination services. This stress can weaken their immune systems, increasing susceptibility to diseases. Probiotics may alleviate stress in bees by modulating their stress responses and supporting overall well-being (Goulson *et al*., 2015). Additionally, probiotics can bolster the immune system, making bees more resilient to pathogens, including bacteria, viruses, and fungi, which contribute to colony collapse disorder (Motta *et al*., 2022). Furthermore, probiotics may improve foraging efficiency by optimizing nutrient absorption and utilization, enhancing the breakdown of pollen and nectar, and consequently increasing the availability of essential nutrients for healthier and more productive bees (Iorizzo *et al*., 2022).

There are number of commercial probiotic products containing mixture of various probiotic bacteria. Effective microorganisms (EM) are probiotic products containing 70–80 varieties of multi-strain beneficial bacteria, including LAB species and *Bifidobacterium*, as well as other microorganisms such as *Aspergillus oryzae*, *S. cerevisiae*, *B. subtilis*, and *Candida glabrata* (Walkunde *et al*., 2011; Aly *et al*., 2016; Laskowska *et al*., 2019) and have demonstrated positive effects in aquaculture, agriculture, animal husbandry, and environmental purification. EM can be applied through water integration or as a feed additive. In aquaculture, EM improves water quality by preventing sludge accumulation in aquafarm ponds (Lananan *et al*., 2014; Zhang *et al*., 2022).

In honeybees, EM probiotics have significantly reduced the total spore count of *Nosema* spp. and improved colony strength (Tlak Gajger *et al*., 2020). While EM shows promise as a probiotic supplement in various sectors, further research is needed to explore its effects on honeybees. This study utilized three commercially available probiotic products TS6 Probiotic (TS6), Vitality Probiotic (VP), and Multi-Defense Probiotic (MDP) designed for human consumption, containing mixtures of probiotic bacteria, on honeybees through direct feeding and their effectiveness was compared with EM probiotics. We hypothesize that direct administration of LAB to honeybees will increase the probiotic LAB population in their gut, thereby enhancing bee strength, improving brood survivorship, productivity, and the overall performance of the colony.

MATERIALS AND METHODS

1. Probiotics

This study utilized three commercially available probiotics: TS6 Probiotic (TS6), Vitality Probiotic (VP), and Multi-Defense Probiotic (MDP) for honey collection, pollen collection, and royal jelly production. Additionally, Effective Microorganisms (EM) probiotic, along with

Table 1. Items used during study and their details

Item	Manufacturer	LAB strains
TS6 Probiotic	Tensall Bio-Tech Co., Ltd., I-Lan, Taiwan	*Bifidobacterium bifidum*, *B. longum*, *B. infantis*, *Lactobacillus casei*, *L. acidophilus*, *L. lactis*
VP Probiotic	Probi AB, Ideongatan, Lund, Sweden	*L. acidophilus*, *B. longum*, *L. paracasei*, *L. rhamnosus*, *L. fermentum*, *L. helvetics*, *Streptococcus thermophilus*, *Kluyveromyces fragilis*, *Bacillus coagulans*
Multi-Defense Probiotic	Feng Hua Biotech Co. LTD., Tainan, Taiwan	*L. paracasei*, *L. rhamnosus*, *L. acidophilus*, *L. gasseri*, *L. johnsonii*
EM Probiotics	EM Research Organization, Okinawa, Japan	Mixture of numbers of beneficial microorganisms

TS6 and VP, was tested at varying dosages to evaluate its effects on bee brood survivorship. These probiotics were selected for their content of LAB, which are recognized for their probiotic potential in the honeybee industry. Detailed product information is provided in Table 1.

To activate the EM probiotics, methods reported by Lananan *et al*. (2014) and Kaur *et al*. (2024) were employed. A total of 1 g of EM was added to a 3 kg bucket, followed by the addition of 2 L of distilled water and 30 g of refined white sugar. The mixture was stirred thoroughly and covered, then stored at a temperature of 38 ± 2°C for seven days. After this period, the pH of the activated EM mixture reached 3.5 ± 0.5, indicating readiness for use. Each hive designated for EM treatment received 10% EM probiotic, which amounted to 50 mL of the prepared mixture mixed with 450 mL of sugar syrup. EM is a liquid concentrate containing effective microorganisms that remain dormant until activated. The presence of a suitable food source or nutrients triggers their activation (Nathaniel *et al*., 2020). The effectiveness of EM can vary depending on the activation process, environmental conditions, and the specific materials used during activation (Liu *et al*., 2023). Therefore, the activation process of EM is crucial for maintaining consistent quality and effectiveness. During the activation process, we followed to the procedures as outlined on the product label to maintain the microbial composition of the final mixture.

2. Apiaries

This study was conducted at three distinct locations in Taiwan. The Honey collection and royal jelly production studies were conducted at Yong Nong Apiary (Da Shu District, Kaohsiung County, Taiwan) while pollen collection was conducted at an apiary at the Aa Tian Apiary (Liu Ying District, Tainan County, Taiwan). The brood survivorship study was carried out at the NPUST apiary located at the National Pingtung University of Science and Technology (NPUST) (Lao Pi District, Pingtung county, Taiwan). Daily weather data, including temperature, relative humidity, rainfall, and wind speed, were obtained from the Central Weather Bureau of Taiwan. All hives in all three apiaries were maintained under consistent conditions throughout the study period. The apiaries housed Italian honeybees (*Apis mellifera ligustica*) in Langstroth beehives (47 cm × 41 cm × 25 cm), each containing eight frames (23.5 cm × 44.5 cm) with an estimated 16,000 worker bees. Beehives were spaced 0.5 meters apart.

All three apiaries have been established for more than five years. The bee colonies have been consistently managed according to the standard beekeeping practices adopted by local beekeepers. These practices include mite control conducted twice a year, specifically in June and November, as well as providing a 50% sugar syrup feed during prolonged rainy periods and typhoons. Additionally, queens are replaced annually at the beginning of the autumn season in October. The bees are housed in standard single-chamber Langstroth hives, which accommodate a maximum of eight frames.

One week prior to the study, honey bee colonies were standardized to ensure uniformity in the number of worker bees (approximately 2,500 worker bees/frame) and the brood area (50%) using the visual Liebefeld estimation method (Gerig, 1983; Bhusal *et al*., 2011; Dainat *et al*., 2020).

3. Honey collection

Honey harvesting by beekeepers in Taiwan typically occurs during the spring season, from late February to the end of April. Therefore, this study was conducted in March 2021. Seasonal plants, such as lychee and longan, bloom during this period, providing ample nectar and pollen for honey production (Kafle and Chinkangsadarn, 2022). The honey collection studies were conducted at Yong Nong Apiary (Da Shu District, Kaohsiung County, Taiwan). The Yong Nong apiary had temperatures ranging from 23.8 to 27°C, relative humidity between 55% and 84%, and an average wind velocity of 12.2 km/h.

The commercially available probiotics (TS6, VP and MDP) were packaged in sachets containing granular powder. Each test item was divided into three doses (0.4 g, 1.2 g, and 2 g per hive) to assess their impact on honey production of bees. Treatments at different doses were mixed in plastic container with 20 mL of water and then combined with 480 mL of sugar syrup to prepare a total volume of 500 mL, which was administered to the bees using feeder trays. Control hives received only 500 mL of sugar syrup. The bees were fed probiotics once a week for four weeks, with feeding stopped one week prior to the honey harvest during this study. Each treatment was applied in six bee hives, each containing eight frames of worker bees.

Standard honey harvesting procedures used by local beekeepers were followed, with honey collected weekly for a total of three harvests for this study. Honey from each hive was collected separately according to the treatments applied, and each frame was returned to its respective hive.

4. Royal jelly production

The preparation processes for the probiotics mixture were similar to those used in honey harvest studies. However, the bees were fed probiotics once a week for four weeks prior to the start of the experiment and continued to feed probiotics until the study was completed. Each treatment was applied in six bee hives, each containing eight frames of worker bees. The royal jelly production studies also were conducted at Yong Nong Apiary (Da Shu District, Kaohsiung County, Taiwan), over a total of three weeks, with data collected at three-day intervals. In the selected hives, the queen bee was located and separated from the experimental frames using a queen excluder. Each hive was then provided with a royal jelly production frame, which consisted of removable bars with thirty-two artificial cells arranged side by side. Each frame contained two bars, totaling 64 queen cells, and was labeled according to the treatment applied. The royal jelly frame was inserted into the production area of the hive after grafting larvae into each cell.

Each artificial cell received 0.01 g of pre-collected royal jelly. Following this, first instar larvae, collected from non-experimental hives, were grafted into each cell using a grafting tool. This process was repeated until all queen cells were filled. After adding the new larvae, the frames were returned to the production area of their respective hives.

Royal jelly was harvested following standard procedures used by local beekeepers, three days after grafting the larvae. The royal jelly was collected according to the treatments applied and weighed every three days until a total of six data sets were collected. During the study period, Yong Nong apiary experienced average temperatures ranging from 28 to 32°C, relative humidity levels of 65% to 87%, and an average wind speed of 12 km/h.

5. Pollen collection

The preparation processes for the probiotics mixture were similar to those used in honey harvest studies, while the feeding of probiotics resembled methods used in royal jelly production experiments. The bees were fed probiotics once a week for four weeks before the experiment began and continued to provide them throughout the study. Each treatment was applied to six bee hives, each containing eight frames of worker bees. Pollen collection by honeybees typically occurs during the spring season in Taiwan when flowers bloom (Kafle and Chinkangsadarn, 2022). This study was conducted in January 2022 at Aa Tian Apiary (Liu Ying District, Tainan County, Taiwan), surrounded by mustard fields that contributed to abundant pollen availability. A standard method used by local beekeepers and also described by Hoover and Ovinge (2018) was employed, using pollen traps placed at the hive entrances.

Each experimental hive was equipped with a pollen trap added every morning during the experimental period.

The collection period spanned from 9 AM to 1 PM, when foraging bees are most active. During four hours, the pollen traps were collected, and pollen were weighed. Data on pollen collection were recorded every two days over a total duration of two weeks. Probiotic treatments mixed in sugar syrup were provided one day prior to pollen harvesting. The average temperature in the apiary throughout the experiment ranged from 24 to 28°C, with relative humidity between 60% to 80% and an average wind velocity of 10 km/h.

6. Bee brood survivorship

The preparation processes for the probiotics mixture were similar to those employed in pollen collection studies. The bees were fed probiotics once a week for four weeks prior to the initiation of the experiment and continued to feed them throughout the study. Each treatment was applied to six bee hives, with each hive containing eight frames populated by worker bees. The brood survivorship studies were performed during the final phase of the study. In these tests, we assessed two probiotic products in comparison to a negative control. Additionally, to strengthen the experimental design, EM probiotics were incorporated as a positive control. Based on results from honey harvest, royal jelly production and pollen collection studies, only commercial probiotics TS6 and VP were further evaluated for bee brood survivorship study as the primary treatments at doses of 0.4 g and 1.2 g per hive. Additionally, a 10% EM probiotic was used as a positive control, along with a negative control to compare the survival rates of broods across different treatments. The bee brood survival study was conducted at the NPUST apiary in Pingtung county from February to March 2022. The average temperature at the NPUST apiary ranged from 22 to 29°C, with relative humidity levels between 61% to 86% and an average wind speed of 11 km/h.

Two frames from each hive were selected based on the availability of broods, and frames were labeled according to their treatment and hive number. These frames were then brought to the observation station for brood survival evaluation. A transparent PVC plastic sheet (23.5 cm × 44.5 cm) was placed flat against the frame (area of comb). The hive number, frame number, and frame side were marked on the sheet.

Three developmental stages—egg, larva, and pupa—were identified and marked from each hive. For the egg survivorship study, fifty cells containing eggs were selected, and transparent PVC sheets were placed over the frames. A dot was placed on each selected egg, and outlines of the fifty egg cells were traced onto the PVC sheet. The same procedure was repeated for identifying pupae and one-day-old larvae, with frames returned to their hives after data collection.

The development of bees at various stages was assessed by observing the growth of eggs, larvae, and pupae as they progressed to subsequent stages in all fifty cells. Marked egg cells were checked to confirm that eggs developed into larvae. Empty marked egg cells were recorded as dead, while larvae and pupae progressing to the next stages were noted as having survived. The total number of surviving brood cells was recorded for each hive. This process was repeated every three days until all adult bees emerged from the marked cells. The following equation (1) was used to estimate the survival rate of the brood.

$$\text{Survivorship}\,(\%) = \frac{\text{Total number of bees survived}}{\text{Sample size}} \times 100\% \quad (1)$$

DATA ANALYSIS

The means of different treatments and doses used in this experiment were compared using one-way Analysis of Variance. To determine significant differences among the means, Tukey's Honestly Significant Difference test was applied at a significance level of $p < 0.05$. Each treatment was replicated six times, and the results are presented as means with standard errors.

RESULTS

1. Honey production

Hives supplemented with TS6 demonstrated significantly higher honey production at a dose of 1.2 g/hive compared to all other treatments and the control group (0 g) ($p < 0.01$, $F = 41.58$, df = 3) (Table 2). Among hives supplemented with various doses of VP, the 2 g/hive dose was identified as optimal, resulting in significantly high-

Table 2. Effects of different probiotics on the total honey production under field the conditions

Treatments	Honey produced (kg/hive) (Mean ± SE)		
	TS6	VP	MDP
Control (0 g)	16.73 ± 0.73c	16.73 ± 0.73c	16.73 ± 0.73c
0.4 g	19.40 ± 0.50b	18.15 ± 0.80bc	22.49 ± 0.61a
1.2 g	22.28 ± 0.44a	19.75 ± 0.34ab	21.96 ± 0.62a
2.0 g	14.88 ± 0.16d	20.86 ± 0.39a	18.85 ± 0.62b

Values in the same column followed by different letters respectively are significantly different at $p < 0.05$. TS6 = TS6 Probiotic, VP = Vitality Probiotic, MDP = Multi-Defense Probiotic.

Table 3. Effects of different probiotics on the royal jelly production under field the conditions

Treatments	Royal jelly (g/hive) (Mean ± SE)		
	TS6	VP	MDP
Control (0 g)	153.75 ± 3.54b	153.75 ± 3.54c	153.75 ± 3.54b
0.4 g	193.00 ± 3.58a	176.50 ± 3.75b	168.50 ± 0.50a
1.2 g	191.50 ± 1.71a	197.75 ± 2.22a	165.00 ± 1.35a
2.0 g	162.50 ± 3.07b	185.00 ± 5.31b	155.75 ± 1.25b

Values in the same column followed by different letters respectively are significantly different at $p < 0.05$. TS6 = TS6 Probiotic, VP = Vitality Probiotic, MDP = Multi-Defense Probiotic.

er honey production compared to all other treatments and the control group (0 g) ($p < 0.01$, $F = 9.11$, df = 3). However, no significant differences in total honey production were observed between hives supplemented with VP at 2 g/hive and 1.2 g/hive or 0.4 g/hive and the control group (0 g) (Table 2).

Similarly, hives supplemented with MDP at a dose of 0.4 g/hive produced significantly more honey than those supplemented with 1.2 g/hive of MDP ($p < 0.01$, $F = 17.45$, df = 3). However, the total honey production between hives supplemented with 0.4 g/hive and 1.2 g/hive of MDP did not differ significantly (Table 2).

When comparing various treatments at different doses for honey production, hives supplemented with MDP at 0.4 g/hive produced significantly more honey than those supplemented with 0.4 g of TS6 and VP ($p < 0.01$, $F = 11.75$, df = 2). However, no significant differences were found between the total honey production of hives supplemented with 0.4 g/hive of TS6 and VP (Table 2).

Moreover, the 1.2 g/hive dose of TS6 resulted in significantly higher honey production compared to VP at the same dose ($p < 0.01$, $F = 8.16$, df = 2). However, no significant difference was observed in total honey production between hives supplemented with 1.2 g/hive of TS6 and VP (Table 2).

Finally, the 2 g/hive dose of VP resulted in significantly higher honey production compared to all other treatments applied ($p < 0.01$, $F = 49.34$, df = 2) (Table 2).

2. Royal jelly production

Hives supplemented with TS6 exhibited optimal royal jelly production at a dose of 0.4 g/hive compared to the 2 g/hive TS6 treatment and the control group (0 g) ($p < 0.01$, $F = 42.55$, df = 3). However, the total royal jelly produced by hives supplemented with TS6 at 0.4 g/hive, 1.2 g/hive, 2 g/hive, and the control group did not differ significantly from one another (Table 3).

Similarly, in hives treated with various doses of VP, the 1.2 g/hive dose resulted in significantly higher royal jelly production compared to all other treatments tested ($p < 0.01$, $F = 24.49$, df = 3). However, no significant difference was observed in royal jelly production between hives supplemented with VP at 0.4 g/hive and 2 g/hive (Table 3).

The hives supplemented with MDP at three different doses displayed trends similar to those of the TS6-supplemented hives. MDP also showed optimal royal jelly production at the 0.4 g/hive dose when compared to the 2 g/hive TS6 treatment and the control group (0 g) ($p < 0.01$, $F = 12.51$, df = 3). However, the total royal jelly produced by hives supplemented with MDP at 0.4 g/hive, 1.2 g/hive, 2 g/hive, and the control group (0 g) did not differ significantly from one another (Table 3).

When comparing the total weight of royal jelly produced across different treatments at varying doses, hives supplemented with TS6 at 0.4 g/hive yielded significantly more royal jelly than those supplemented with 0.4 g/hive of MDP and VP ($p < 0.01$, $F = 17.24$, df = 2). No significant difference was found between the MDP and VP treatments at the 0.4 g/hive dose (Table 3).

Furthermore, the VP treatment at 1.2 g/hive produced significantly more royal jelly compared to both TS6 and MDP at the same dose. This trend continued at the 2 g/hive dose, with VP again showing significantly higher royal jelly production than both TS6 and MDP (1.2 g/hive: $p < 0.01$, $F = 151.68$, df = 2; 2 g/hive: $p < 0.01$, $F =$

17.98, df = 2). No significant difference was observed in terms of royal jelly production between the TS6 and MDP treatment groups at the 2 g/hive dose (Table 3).

3. Pollen collection

Hives supplemented with TS6 demonstrated optimal pollen collection, with a significant difference observed at a dose of 0.4 g compared to the 1.2 g/hive and 2 g/hive doses of TS6, as well as the control group (0 g) ($p < 0.01$, $F = 32.96$, df = 3). However, no significant differences in pollen collection were found between the TS6 doses of 1.2 g/hive and 2 g/hive (Table 4).

In contrast, hives supplemented with various doses of VP did not show any improvement in pollen collection compared to the control group (0 g). Interestingly, the control group exhibited significantly higher pollen collection results than all VP treatments ($p < 0.01$, $F = 12.41$, df = 3) (Table 4).

Hives supplemented with 2 g/hive of MDP collected significantly more pollen than all other treatments, including the control group (0 g) ($p < 0.01$, $F = 24.04$, df = 3). However, no significant differences were observed in pollen collection among the TS6 doses of 0.4 g/hive, 1.2 g/hive, and the control group (0 g) (Table 4).

Furthermore, hives supplemented with TS6 at doses of 0.4 g/hive, 1.2 g/hive, and 2 g/hive showed significantly higher pollen collection compared to the other two probiotics tested (0.4 g/hive: $p < 0.01$, $F = 868.38$, df = 2; 1.2 g/hive: $p < 0.01$, $F = 87.04$, df = 3; 2 g/hive: $p < 0.01$, $F = 135.14$, df = 3) (Table 4).

4. Survivorship of broods

To evaluate the effects of probiotics on brood growth and development, particularly the survivorship of each developmental stage (egg, larva, and pupa), two doses (0.4 g/hive and 1.2 g/hive) of TS6 and VP were assessed and compared with a 10% EM probiotic and control groups (0 g). These doses were selected based on previous productivity studies involving honey, pollen, and royal jelly.

At the conclusion of the study, 18 days after treatment (DAT), 80% of eggs in hives treated with 1.2 g/hive of TS6 survived and developed into adults. This survival rate was significantly higher than that of all other treatments evaluated ($p < 0.01$, $F = 10.58$, df = 5). However, no significant differences were observed in egg survival rates among hives supplemented with EM probiotic, VP at 0.4 g/hive, VP at 1.2 g/hive, or the control group (0 g), nor between VP at 0.4 g/hive and TS6 at 0.4 g/hive (Table 5).

At 18 DAT, 94.5% of larvae in hives treated with 0.4 g/hive of TS6 survived and developed into adults, representing a significantly higher survival rate compared to all other treatments ($p < 0.05$, $F = 1.79$, df = 5). However, no significant differences were noted in larval survival rates among hives supplemented with VP at 1.2 g/hive, the control group (0 g), EM, VP at 0.4 g/hive, or TS6 at 1.2 g/hive (Table 5).

At 18 DAT, 95% of pupae in hives treated with 0.4 g/hive of TS6 survived and developed into adults, which was significantly higher than the survival rates observed in all other treatments ($p < 0.01$, $F = 7$, df = 5). Nonetheless, no significant differences were found in pupal survival rates among hives supplemented with EM, VP at 0.4 g/hive, TS6 at 1.2 g/hive, the control group (0 g), or between VP at 1.2 g/hive and TS6 at 0.4 g/hive (Table 5).

Table 4. Effects of different probiotics on the total pollen collection under field the conditions

Treatment	TS6	VP	MDP
Control (0 g)	458.67 ± 22.73c	458.67 ± 22.73a	458.67 ± 22.73a
0.4 g	621.67 ± 1.33a	373.33 ± 4.37b	505.33 ± 5.69a
1.2 g	536.33 ± 2.31b	349.33 ± 19.81b	492.67 ± 3.53a
2.0 g	564.33 ± 5.78b	386.33 ± 0.88b	351.00 ± 16.00b

Values in the same column followed by different letters respectively are significantly different at $p < 0.05$. TS6 = TS6 Probiotic, VP = Vitality Probiotic, MDP = Multi-Defense Probiotic.

Table 5. Effects of different probiotics on the survivorship of eggs, larvae and pupae of honeybees under field the conditions

Developmental stage	Survival rates (%)		
	Eggs	Larvae	Pupae
Control (0 g)	52.50 ± 0.50c	75.00 ± 1.92c	86.00 ± 0.00b
EM (10%)	49.50 ± 1.50c	88.00 ± 3.56b	88.00 ± 1.63b
TS6 (0.4 g/hive)	68.00 ± 1.41b	94.50 ± 2.74a	95.00 ± 2.65a
TS6 (1.2 g/hive)	80.00 ± 3.27a	82.00 ± 9.42b	85.00 ± 3.00b
VP (0.4 g/hive)	58.00 ± 7.44bc	86.00 ± 2.00b	84.50 ± 1.50b
VP (1.2 g/hive)	53.50 ± 2.50c	70.00 ± 12.28c	94.50 ± 1.71a

Values in the same column followed by different letters are significantly different at $p < 0.05$. EM = Effective microorganisms, TS6 = TS6 Probiotic, VP = Vitality Probiotic.

DISCUSSION

In this study, we observed that hives supplemented with probiotics (TS6, VP, and MDP) produced significantly higher amounts of honey compared to hives that did not receive any probiotic supplements. Pătruică and Hutu (2013) also reported increased honey production in honeybee colonies supplemented with LAB. Similarly, Audisio *et al*. (2011) found a significant increase in honey production when honeybee colonies were treated with *Lactobacillus johnsonii* CRL1647. The probiotics used in this study, including TS6, VP, and MDP, also contain various species and strains of LAB. Thus, our findings are consistent with earlier studies.

Probiotics can reduce the overall concentration of pathogens in the bee gut (Pătruică and Hutu, 2013). Additional supplementations of probiotic bacteria may enhance digestion and nutrient assimilation, ultimately strengthening honeybee health. Reduced pathogenic bacteria levels can lead to an increase in beneficial bacteria, which improves resilience against diseases (Pătruică and Mot, 2012). Beneficial bacteria possess antimicrobial properties that can prevent gastrointestinal infections. Tejero-Sariñena *et al*. (2012) suggested that beneficial bacteria produce organic acids that lower the intestinal pH in bees, further suppressing pathogen growth. Additionally, in strong colonies, increased oviposition by queen bees can lead to higher honeybee populations and, consequently, more honey production (Hernández-García *et al*., 2021). Each probiotic product used in this study significantly enhanced honey production; however, due to differences in LAB species concentrations, and especially prepared for human consumptions, each probiotic may function differently. Notably, a dose of 1.2 g/hive was optimal for enhancing honey production with TS6, VP, or MDP.

The results of this study indicated that probiotic supplementation significantly improved the quantity of royal jelly produced. As discussed, probiotics in the gut microbiota of bees can enhance nutrient and mineral digestion, strengthening the bees. Royal jelly is primarily produced by the mandibular and hypopharyngeal glands of honeybees. Shawer and Mousa (2016) observed an increase in the surface area of these glands when honeybees were fed a protein source compared to those lacking protein in their diet. Protein plays a crucial role in the development of muscles and glands in honeybees. We hypothesize that improved digestion in the honeybee gut, following probiotic supplementation, may enhance protein digestion. In contrast, Kaznowski *et al*. (2005) did not observe changes in protein concentration when comparing the guts of bees with high and low concentrations of beneficial bacteria.

In terms of pollen collection, the amount of pollen gathered after supplementation with TS6 at all three application doses was significantly higher than that of other tested treatments. Forager bees are responsible for collecting pollen and nectar, and various external factors—such as climate, season, temperature, flowering, and pests—can limit their collection efforts (García-García *et al*., 2001). Pollen is typically gathered during the spring when flowers are in full bloom. Internal factors, including disruptions in bee growth and health, may also affect pollen collection.

Royal jelly is primarily produced by the mandibular and hypopharyngeal glands (HPG) of young worker bees (Hasan *et al*., 2022). Hassan and Elenany (2024) reported a significant increase in the morphometric parameters of HPGs in bees fed probiotics compared to those given only sugar syrup. Larger HPGs are more capable of producing royal jelly than smaller ones. Thus, using probiotics as a natural alternative enhances the development of the HPGs in *Apis mellifera* nursing bees, positively affect the royal jelly production (Hasan *et al*., 2022; Hassan and Elenany, 2024).

Despite this, lower doses of TS6 appeared to enhance pollen collection compared to the control. In contrast, the addition of VP and MDP in the bees' diet negatively affected the amount of pollen collected relative to the control. Each probiotic function differently, and many commercial probiotics are developed specifically for animal and human use (Juhász *et al*., 2019). Applying these probiotics to insects may yield adverse effects, as honeybee gut and microbiota differ from those of humans and animals. Disruption of honeybee gut microbiota can be detrimental, potentially reducing host fitness and increasing mortality rates. In our study, supplementing VP to honeybee colonies may have led to a buildup of nitrogenous waste in the bee gut, suppressing normal functions, similar to findings by Borges *et al*. (2021).

Furthermore, all three products contain the common probiotic bacterial strain *Lactobacillus acidophilus*, while both TS6 Probiotic and VP share the strain *Bifidobacterium longum*. Among these products, VP had the highest number of probiotic strains and was the only one that includes yeast. However, the application of VP and MDP could not enhance the pollen collection.

Pollen collection is primarily influenced by the number of foraging flights and the amount of pollen carried by bees (Fewell and Winston, 1992; Pernal and Currie, 2001). Given that all hives had nearly identical population strengths and the nearest mustard field was within 200 meters, we considered two potential explanations for the lower pollen collection in VP or MDP treated hives compared to the control group.

First, pollen serves as a source of prebiotics and probiotics (Utoiu *et al*., 2018), and the bees' guts are already populated with various microorganisms (Yang *et al*., 2025). The introduction of additional VP or MDP, which contains at least five probiotic strains and yeast, may result in an overload of microbial strains in the bees' guts. This situation could lead to competition among probiotic bacteria (Zhu *et al*., 2010) and result in the release of compounds that could disrupt digestion, potentially leading to fatigue in bees. Second, pollen is a high-protein food. The ingestion of excessive probiotics may increase the bees' hunger, prompting them to feed more protein rich pollen than usual. This overfeeding could result in bees resting more frequently rather than foraging for additional pollen, ultimately leading to reduced pollen collection.

Our study demonstrated that supplementing hives with 1.2 g/hive of TS6 significantly increased brood survivorship from egg to adult honeybee. Additionally, both 0.4 g/hive and 1.2 g/hive doses of TS6 and VP significantly enhanced brood survivorship across all developmental stages. Specifically, TS6 at 0.4 g/hive significantly improved survivorship from larvae to adults compared to other treatments. As previously discussed, probiotics can profoundly impact honeybee gut microbiota, leading to improved digestion, increased nutrient absorption, lowered gut pH, and enhanced growth of beneficial bacteria. Beneficial bacteria are first introduced to the honeybee gut at the pupal stage. Padmashree *et al*. (2020) suggested that high concentrations of beneficial bacteria in the gut microbiota can increase brood comb area within the colony. Elevated levels of beneficial bacteria may also enhance bee immunity and promote the growth of eggs, larvae, and pupae.

CONCLUSION

Probiotic supplementation significantly enhances honeybee productivity. Field studies demonstrated notable increases in royal jelly production with the addition of TS6 at 0.4 g/hive and VP at 1.2 g/hive and 2 g/hive. Similarly, pollen collection was significantly boosted with TS6 at 0.4 g/hive and 1.2 g/hive. Furthermore, honey production saw substantial increases when hives were supplemented with TS6 at 1.2 g/hive, VP at 2 g/hive, or MDP at 0.4 g/hive and 1.2 g/hive. Notably, the application of 1.2 g of TS6 and VP also significantly improved brood survivorship from egg to adult, underscoring the positive effects on the honeybee life cycle. This study confirms that incorporating TS6 into the diet of European honeybees can enhance honey production, royal jelly output, pollen collection, and brood survivorship. For optimal results, it is recommended to supplement TS6 at a dose of 1.2 g/hive in 500 mL of sugar syrup every two weeks. Probiotics present a promising alternative for improving honeybee productivity and colony strength. Further research is essential to explore the full potential of probiotics in enhancing honeybee production.

ACKNOWLEDGEMENTS

We would like to thank Mr. Lu Qing Jing, Simran Kayastha and Sabrina Diana Blanco and the NPUST Bee Team members for their support during this study.

FUNDING

No external funding is involved for this study.

LITERATURE CITED

Aljedani, D. M. 2022. Antibiotic treatment (tetracycline) effect on bio-efficiency of the larvae honey bee (*Apis mellifera jemenatica*). Saudi J. Biol. Sci. 29: 1477-1486.

Aly, H. A., M. Abdel-Rahim, A. M. Lotfy, B. S. Abdelaty and G. R. Sallam. 2016. The applicability of activated carbon, natural zeolites, and probiotics (EM®) and its effects on ammonia removal efficiency and fry performance of european seabass dicentrarchus labrax. J. Aquac. Res. Dev. 7: 1-8.

Anee, I. J., S. Alam, R. A. Begum, R. M. Shahjahan and A. M. Khandaker. 2021. The role of probiotics on animal health and nutrition. J. Basic Appl. Zool. 82: 1-16.

Audisio, M. C., M. J. Torres, D. C. Sabaté, C. Ibarguren and M. C. Apella. 2011. Properties of different lactic acid bacteria isolated from *Apis mellifera* l. bee-gut. Microbiol. Res. 166: 1-13.

Bermudez-Brito, M., J. Plaza-Díaz, S. Muñoz-Quezada, C. Gómez-Llorente and A. Gil. 2012. Probiotic mechanisms of action. A. Nutri. Metabol. 61: 160-174.

Bhusal, S. J., L. Kafle, R. B. Thapa and C.-J. Shih. 2011. Effect of colony strength on the performance of honeybees (*Apis mellifera*) in nepal (Hymenoptera: Apidae). Sociobiology 58(2): 435-447.

Borges, D., E. Guzman-Novoa and P. H. Goodwin. 2021. Effects of prebiotics and probiotics on honey bees (*Apis mellifera*) infected with the microsporidian parasite *Nosema ceranae*. Microorganisms 9: 481.

Dainat, B., V. Dietemann, A. Imdorf and J.-D. Charrière. 2020. A scientific note on the 'liebefeld method' to estimate honey bee colony strength: its history, use, and translation. Apidologie 51(3): 422-427.

de Paula, G. T., C. Menezes, M. T. Pupo and C. A. Rosa. 2021. Stingless bees and microbial interactions. Curr. Opin. Insect Sci. 44: 41-47.

Evans, J. D. and D. L. Lopez. 2004. Bacterial probiotics induce an immune response in the honey bee (Hymenoptera: Apidae). J. Econ. Entomol. 97: 752-756.

Fewell, J. H. and M. L. Winston. 1992. Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. Beh. Ecol. Sociobiol. 30(6): 387-393.

García-García, M. C., P. L. Ortiz and M. J. Díez Dapena. 2001. Pollen collecting behaviour of *Apis mellifera* during one day. Grana 40: 205-209.

Gerig, L. 1983. Lehrgang zur erfassung der volksstarke. Schweizerische Bienenzeitung 106: 199-204.

Goulson, D., E. Nicholls, C. Botías and E.L. Rotheray. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347: 1255957.

Hasan, A., J. I. Qazi, F. Tabssum and A. Hussain. 2022. Feeding probiotics and organic acids to honeybees enhances acinal surface area of their hypopharyngeal glands. Res. Vet. Sci. 149: 47-50.

Hassan, A. A.-M. and Y. E. Elenany. 2024. Influence of probiotics feed supplementation on hypopharyngeal glands morphometric measurements of honeybee workers *Apis mellifera* L. Probiotics Antimicrob. Proteins 16(4): 1214-1220.

Hernández-García, J. E., J. A. Rodríguez Díaz, O. Estrada-Cutiño, Y. Solenzal-Valdivia, K. J. Fernández-León and A. J. Rondón-Castillo. 2021. Potentialities of the utilization of zootechnical additives in Cuban apiculture. Pastos y Forrajes 44: eE14.

Hoover, S. E. and L. P. Ovinge. 2018. Pollen collection, honey production, and pollination services: managing honey bees in an agricultural setting. J. Econ. Entomol. 111: 1509-1516.

Horak, R. D., S. P. Leonard and N. A. Moran. 2020. Symbionts shape host innate immunity in honeybees. Proc. R. Soc. 287: 20201184.

Iorizzo, M., F. Letizia, S. Ganassi, B. Testa, S. Petrarca, G. Albanese, D. Di Criscio and A. De Cristofaro. 2022. Functional properties and antimicrobial activity from lactic acid bacteria as resources to improve the health and welfare of honey bees. Insects 13: 308.

Jasim, M. S. and A. A. Fehan. 2017. Application of probiotic with different levels of citric acid supplementation in the diet for promotes the production efficiency of broiler chickens. ISOR-JAVS 10: 50-57.

Juhász, Á., A. Veress, O. Adamcsik, M. Szabolcs and H. Szalontai. 2019. The impact of inulin as feed supplement on gut microbiota of honey bee (*Apis mellifera*). pp. 78-79. in 18th Alps-Adria Scientific Workshop, ed. by A. Dujmović. Hungarian Academy of Sciences, Cattolica, Italy.

Kafle, L. and S. Chinkangsadarn. 2022. Beekeeping and bee product quality control in Taiwan. J. Entomol. Res. 46: 1069-1077.

Kaur, B., R. Choudhary, G. Sharma and L. K. Brar. 2024. Sustainable and effective microorganisms method for wastewater treatment. Desalin. Water Treat. 319: 100419.

Kaznowski, A., B. Szymas, E. Jazdzinska, M. Kazimierczak, H. Paetz and J. Mokracka. 2005. The effects of probiotic supplementation on the content of intestinal microflora and chemical composition of worker honey bees (*Apis mellifera*). J. Apic. Res. 44: 10-14.

Kešnerová, L., R. A. Mars, K. M. Ellegaard, M. Troilo, U. Sauer and P. Engel. 2017. Disentangling metabolic functions of bacteria in the honey bee gut. PLoS Biol. 15: e2003467.

Lananan, F., S. H. A. Hamid, W. N. S. Din, N. a. Ali, H. Khatoon, A. Jusoh and A. Endut. 2014. Symbiotic bioremediation of aquaculture wastewater in reducing ammonia and phosphorus utilizing effective microorganism (em-1) and microalgae (*Chlorella* sp.). Int. Biodeterior. Biodegrad. 95: 127-134.

Laskowska, E., Ł. S. Jarosz and Z. Grądzki. 2019. Effect of the em bokashi® multimicrobial probiotic preparation on the non-specific immune response in pigs. Probiotics Antimicrob. Proteins 11: 1264-1277.

Lika, E., M. Kostić, S. Vještica, I. Milojević and N. Puvača. 2021. Honeybee and plant products as natural antimicro-

bials in enhancement of poultry health and production. Sustainability 13: 8467.

Liu, X., M. Zubair, L. Kong, Y. Shi, H. Zhou, L. Tong, R. Zhu, Y. Lv and Z. Li. 2023. Shifts in bacterial diversity characteristics during the primary and secondary fermentation stages of bio-compost inoculated with effective microorganisms agent. Bioresour. Technol. 382: 129163.

Mojgani, N., M. Bagheri, S. Ashique, A. Islam, M. Moharrami, H. Modirrousta and A. Hussain. 2024. Honeybee defense mechanisms: role of honeybee gut microbiota and antimicrobial peptides in maintaining colony health and preventing diseases. Microb. Pathog. 198: 107161.

Moritz, R. F., J. De Miranda, I. Fries, Y. Le Conte, P. Neumann and R. J. Paxton. 2010. Research strategies to improve honeybee health in europe. Apidologie 41: 227-242.

Motta, E. V., J. E. Powell, S. P. Leonard and N. A. Moran. 2022. Prospects for probiotics in social bees. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 377: 20210156.

Nathaniel, O., A. R. M. Sam, N. H. A. S. Lim, O. Adebisi and M. Abdulkareem. 2020. Biogenic approach for concrete durability and sustainability using effective microorganisms: a review. Constr. Build. Mater. 261: 119664.

Padmashree, I., S. S. J. Roseleen, C. G. L. Justin and J. Ejilane. 2020. Effect of probiotic supplement on the disease management and brood development of the indian honey bee, *Apis cerana indica* f. (Hymenoptera: Apidae). J. Entomol. Zool. Stud. 8: 496-500.

Pătruică, S. and D. Mot. 2012. The effect of using prebiotic and probiotic products on intestinal micro-flora of the honeybee (*Apis mellifera carpatica*). Bull. Entomol. Res. 102: 619-623.

Pătruică, S. and I. Hutu. 2013. Economic benefits of using prebiotic and probiotic products as supplements in stimulation feeds administered to bee colonies. Turk. J. Vet. Anim. Sci. 37: 259-263.

Pătruică, S., G. Dumitrescu, R. Popescu and N. M. Filimon. 2013. The effect of prebiotic and probiotic products used in feed to stimulate the bee colony (*Apis mellifera*) on intestines of working bees. J. Food Agric. Environ. 11: 2461-2464.

Pernal, S. F. and R. W. Currie. 2001. The influence of pollen quality on foraging behavior in honeybees (*Apis mellifera* L.). Behav. Ecol. Sociobiol. 51(1): 53-68.

Ramos, O. Y., M. Basualdo, C. Libonatti and M. F. Vega. 2020. Current status and application of lactic acid bacteria in animal production systems with a focus on bacteria from honey bee colonies. J. Appl. Microbiol. 128: 1248-1260.

Shawer, D. M. and K. M. Mousa. 2016. Effect of brewer yeast diet on the biological activities and the development of mandibular, hypopharyngeal, and wax glands of honeybees, *Apis mellifera* l. (Hymenoptera: Apidae). Bull. Ent. Soc. Egypt. 42: 13-20.

Tejero-Sariñena, S., J. Barlow, A. Costabile, G. R. Gibson and I. Rowland. 2012. In vitro evaluation of the antimicrobial activity of a range of probiotics against pathogens: evidence for the effects of organic acids. Anaerobe 18: 530-538.

Tlak Gajger, I., J. Vlainić, P. Šoštarić, J. Prešern, J. Bubnič and M. I. Smodiš Škerl. 2020. Effects on some therapeutical, biochemical, and immunological parameters of honey bee (*Apis mellifera*) exposed to probiotic treatments, in field and laboratory conditions. Insects 11: 638.

Utoiu, E., A. Oancea, A.-M. Stanciuc, L. M. Ştefan, A. Toma, A. Moraru, C. F. Diguţa, F. Matei, C. P. Cornea and F. Oancea. 2018. Prebiotic content and probiotic effect of kombucha fermented pollen. AgroLife Sci. J. 7(2): 149-156.

Walkunde, T., S. Adangale and A. Deshmukh. 2011. Effect of effective micro-organism (Probiotics) technology on growth performance of broiler. Indian J. Anim. Res. 45: 318-321.

Yang, C., J. Hu, Q. Su, Z. Zhang, Y. Du, J. Wang, H. Sun, B. Han, J. Tang and L. Guo. 2025. A review on recent taxonomic updates of gut bacteria associated with social bees, with a curated genomic reference database. Insect Sci. 32(1): 2-23.

Zapata-Hernández, G., M. Gajardo-Rojas, M. Calderón-Seguel, A. A. Muñoz, K. P. Yáñez, F. Requier, F. E. Fontúrbel, P. I. Ormeño-Arriagada and H. Arrieta. 2024. Advances and knowledge gaps on climate change impacts on honey bees and beekeeping: a systematic review. Glob. Change Biol. 30: e17219.

Zhang, Z., Y. Wu, Y. Zhang, Y. Cao, S. Chen, Z. Tian, Q. Li, X. Sun and A. Chen. 2022. Effects of adding em bacteria and mechanical aeration on water quality, growth and antioxidant status of meretrix meretrix and exopalaemon carinicauda farmed in the clam-shrimp polyculture system. Aquac. Res. 53: 1823-1832.

Zhu, Y., L. Xiao, D. Shen and Y. Hao. 2010. Competition between yogurt probiotics and periodontal pathogens in vitro. Acta Odontol. Scand. 68(5): 261-268.