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The nutritional needs of stingless bees (*Meliponini*) are critical for their health, productivity, and overall colony success. However, the development of artificial diets for stingless bees is a complex area of research that has garnered attention due to the challenges faced by these bees in natural foraging conditions. This study was conducted to determine the effect of artificial diets on stingless bee (*Tetragonula pagdeni*) in both laboratory and meliponary. For the laboratory experiment, 270 newly emerged *T. pagdeni* were divided into three replicated feeding groups (30 bees/cage). Each feeding group received artificial diets containing probiotics (A3), a commercial diet for honey bees (A2), or negative control (A1), and each group was fed with 50% sucrose solution (w/v) for 30 days. The effect of diets on survival, fat body content, and HPG acini width were measured. The most effective diet will be selected for the field experiment. In the field experiment, 60 stingless bee (*T. pagdeni*) colonies were divided into two feeding groups replicated at three apiary sites. Feeding groups received diets or negative control. Diets were analyzed for macronutrient content and then evaluated concerning life span, hypopharyngeal gland size, fat body content, colony population size, and honey production. The results demonstrated that an artificial diet A3 containing probiotics increases the protein content, which benefits stingless bee workers by increasing lifespan, fat body content, and HPG size. In the field test, diet A3 successfully increased the colony size, including eggs, pollen pot, and honey production, about 3 times compared to the control without the colony's collapse. Our findings highlight the important and beneficial effects of an artificial diet for physiological development and health in stingless bees and reveal that probiotics are the key factor governing HPG size and lifespan in worker bees. Besides, the artificial diets were also effective in increasing honey production and improving colony fitness. The data provide a foundation for future studies to investigate the impact of supplements for meliponiculture to mitigate the impacts of food shortages due to climate change, which will help farmers reduce losses and generate income without compromising the quality and safety of honey.



The effects of supplementary diets on Thai commercial stingless bee (*Tetragonula pagdeni*)

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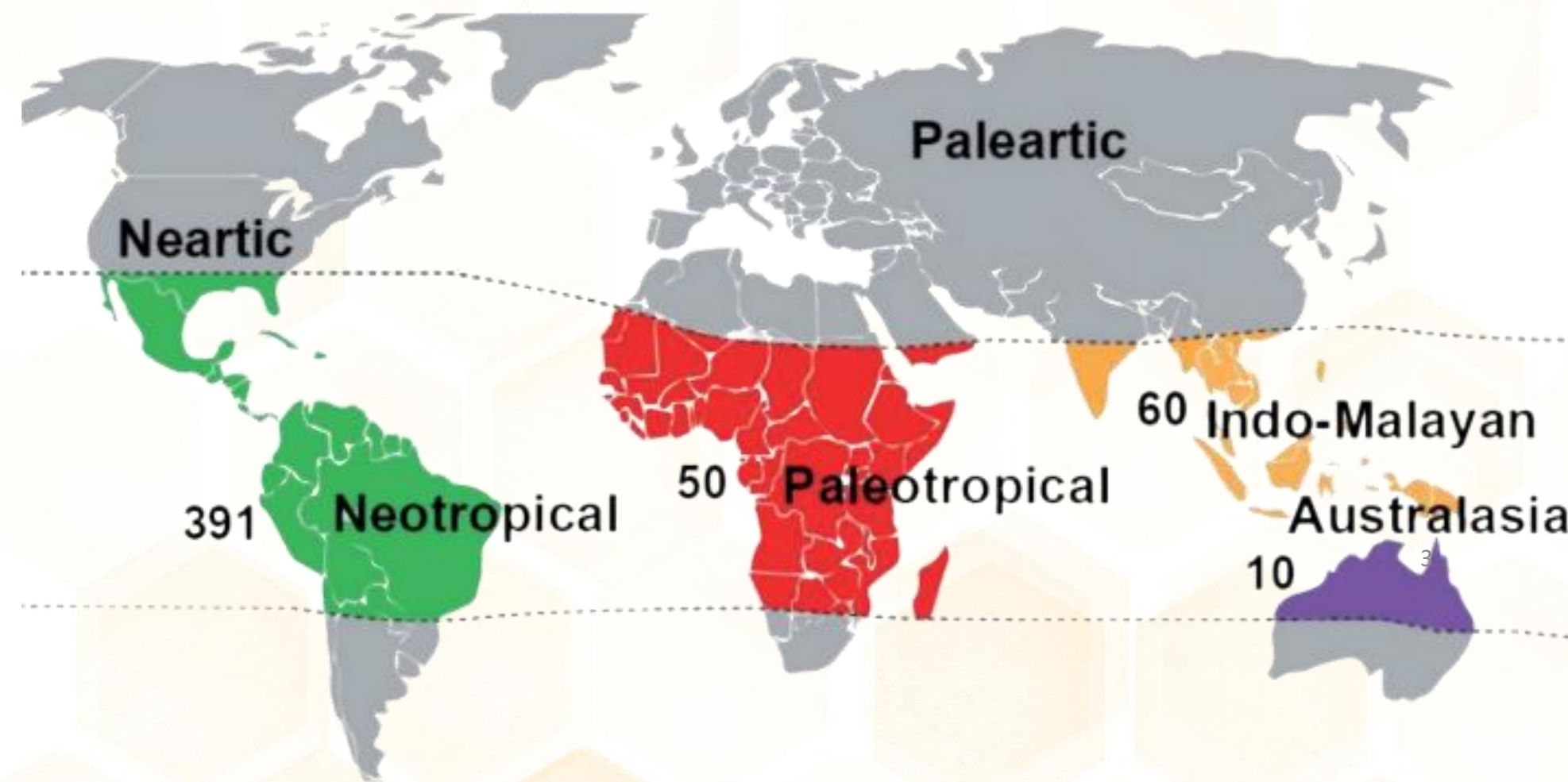
The effects of supplementary diets on Thai commercial stingless bee (*Tetragonula pagdeni*)

Jakkrawut Maitip

King Mongkut's University of Technology North Bangkok, Thailand



The distribution of stingless bee



Worldwide

> 500 species

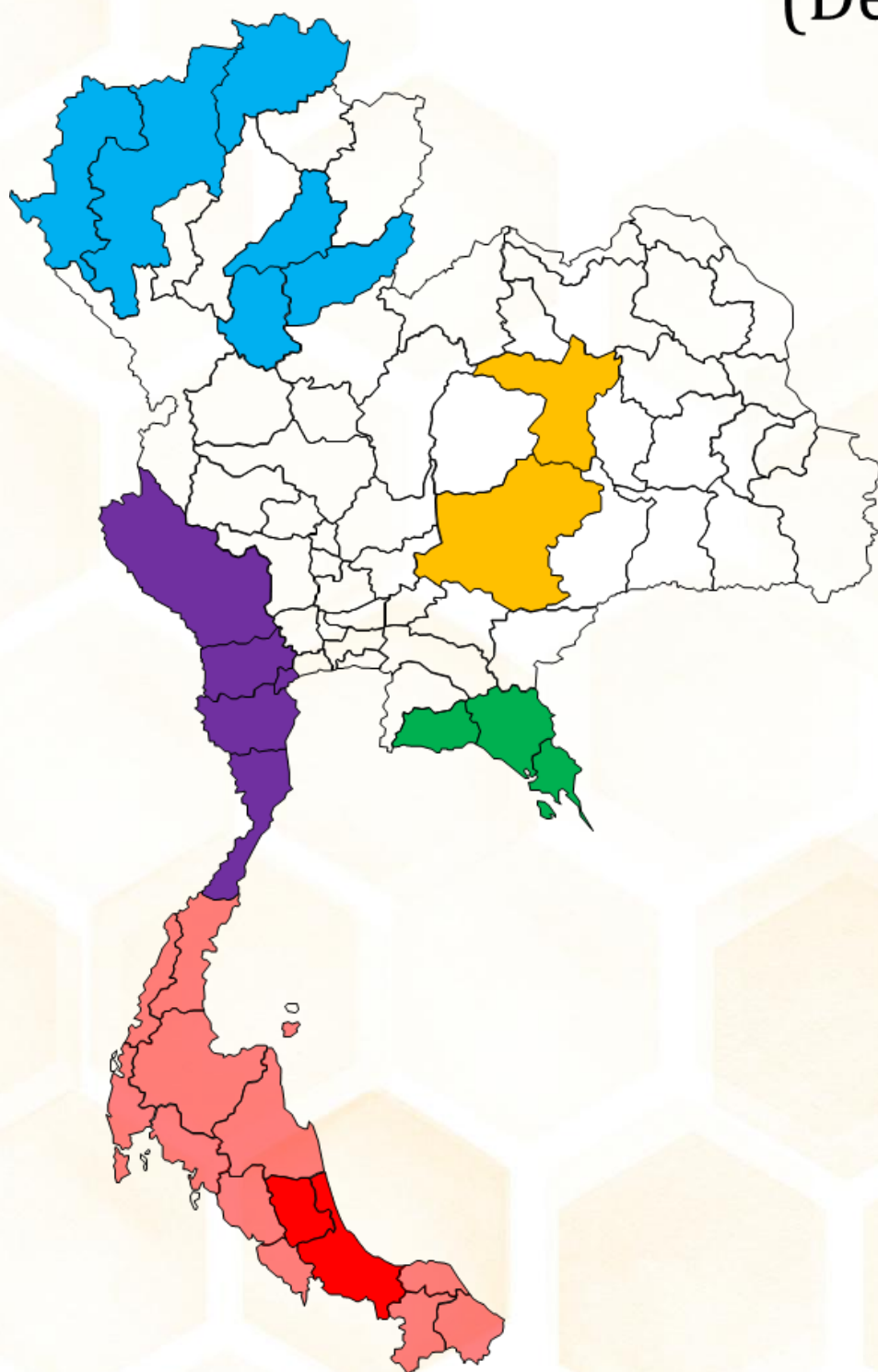


Thailand

~ 40 species

Meliponiculture in Thailand

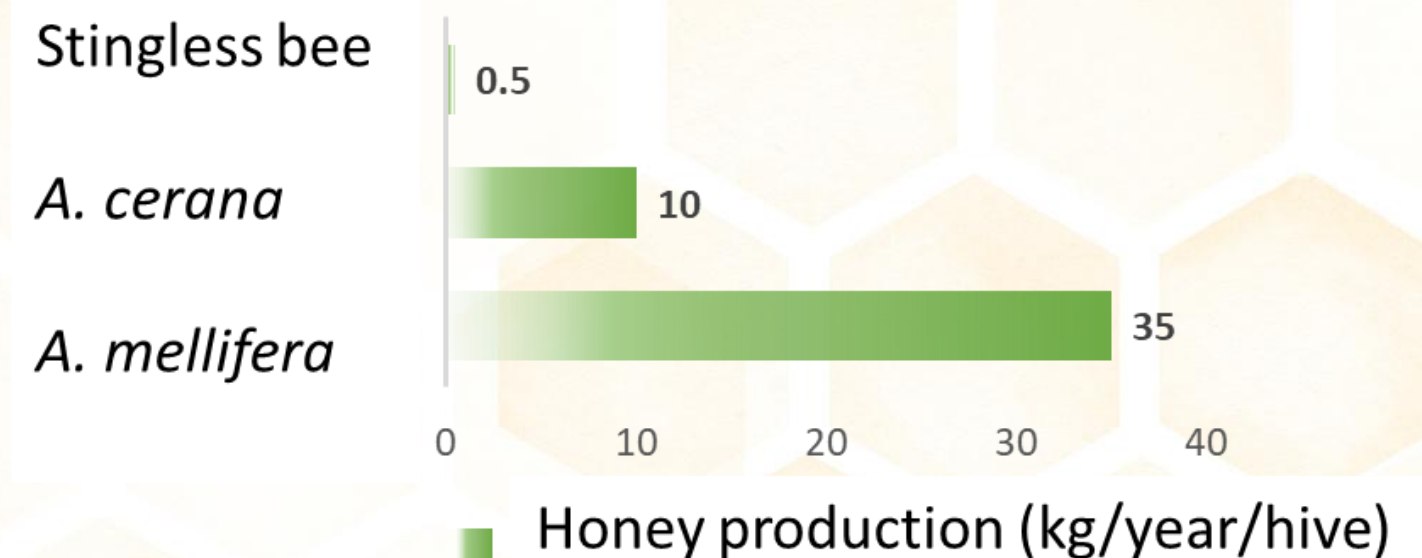
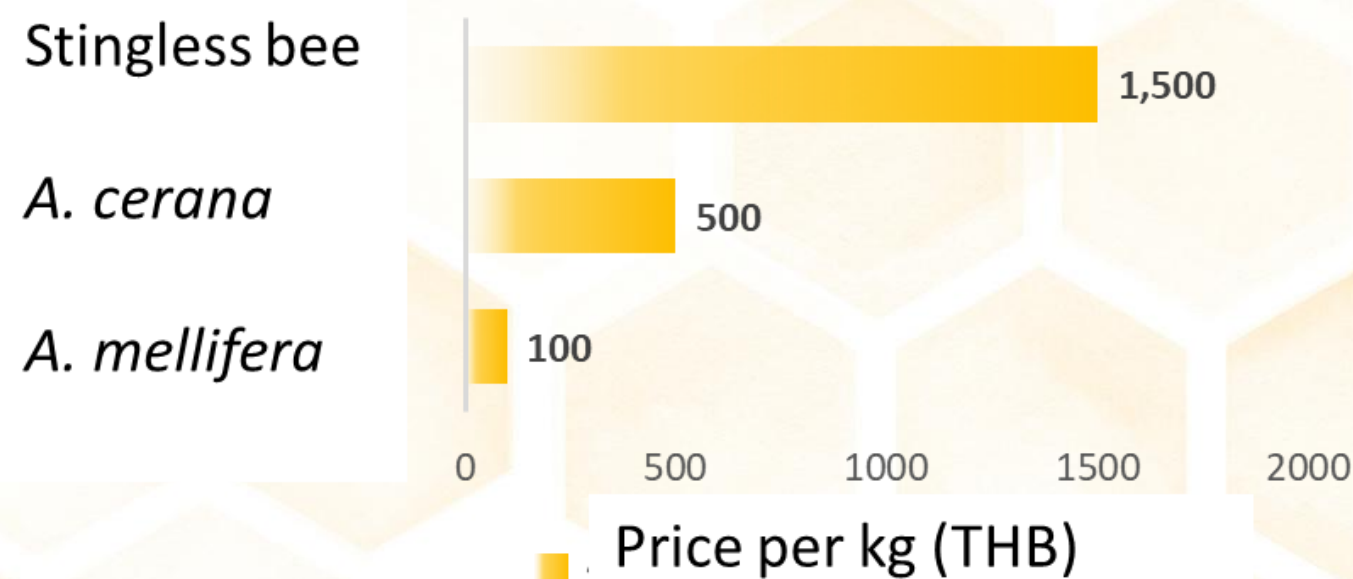
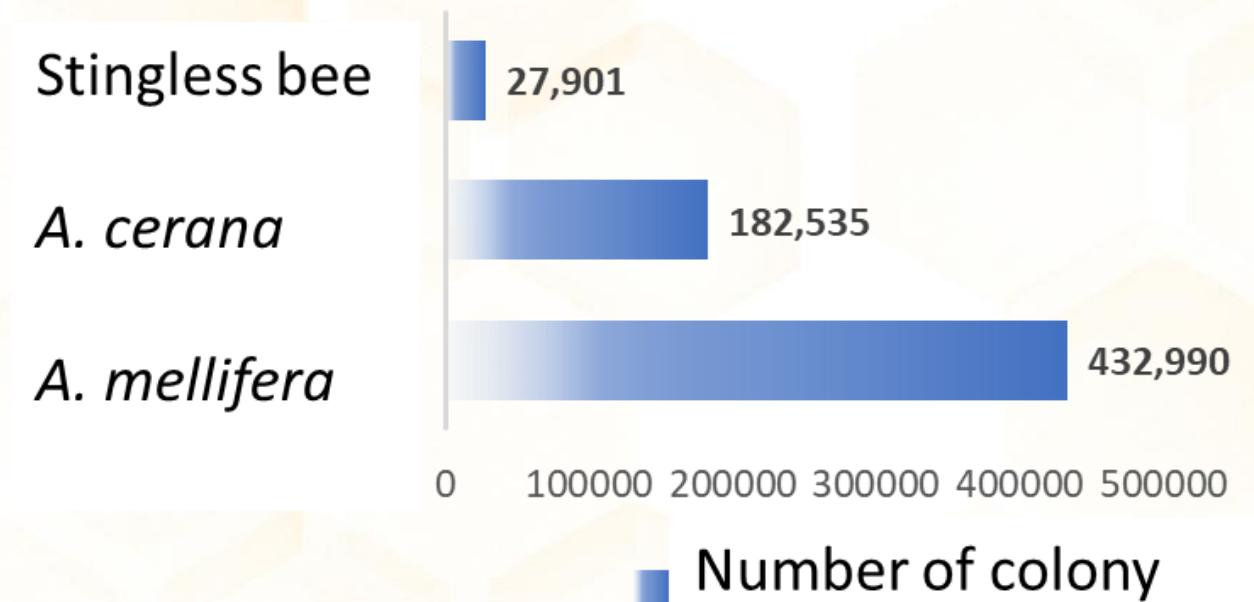
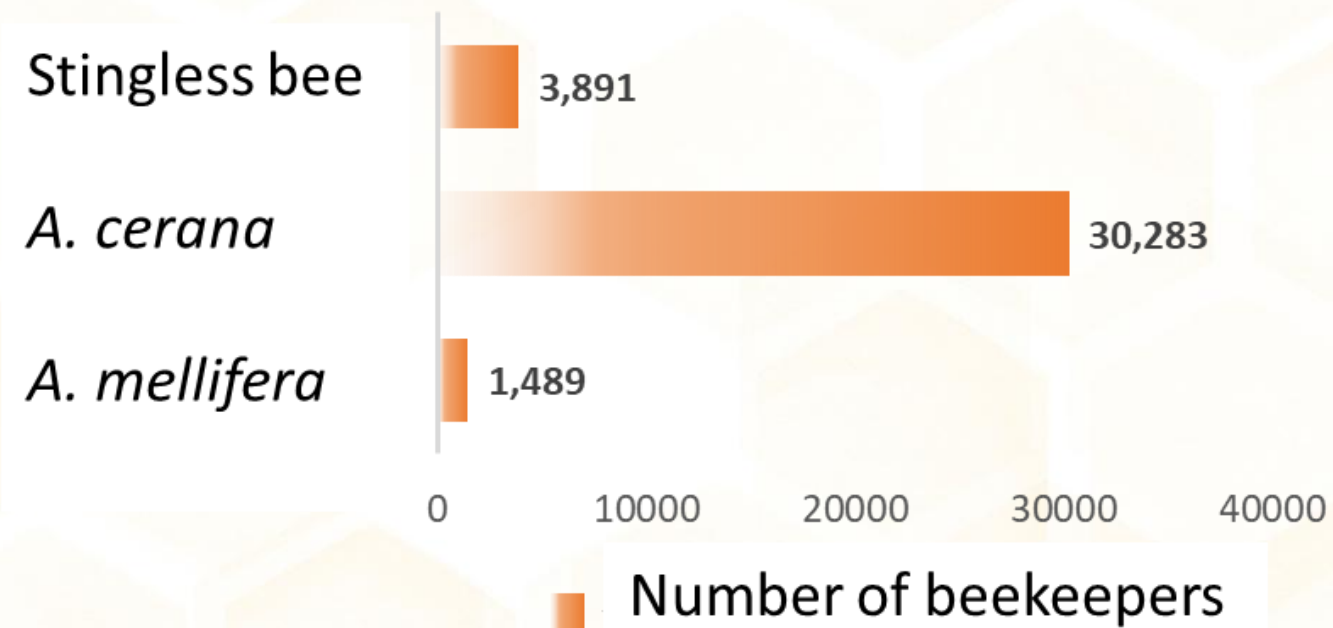
(Department of Agriculture Extension, 2022)



	2014	2022
No. of stingless beekeeper	700	3,890
No. of stingless bee (hive)	5,000	27,900
No. of hive/farm	10-50	10 - >500
honey production (ton/year)	-	12.24

Comparative data on three main honey producers in Thailand

(Department of Agriculture Extension, 2022)



Northern

- *Tetragonula laeviceps*
- *****Tetragonula pagdeni*****

Northeast

- *****Tetragonula pagdeni*****

Eastern

- *****Tetragonula pagdeni*****

Southern

● ***Heterotrigona itama***

● ***Geniotrigona thoracica***

● *****Tetragonula pagdeni*****

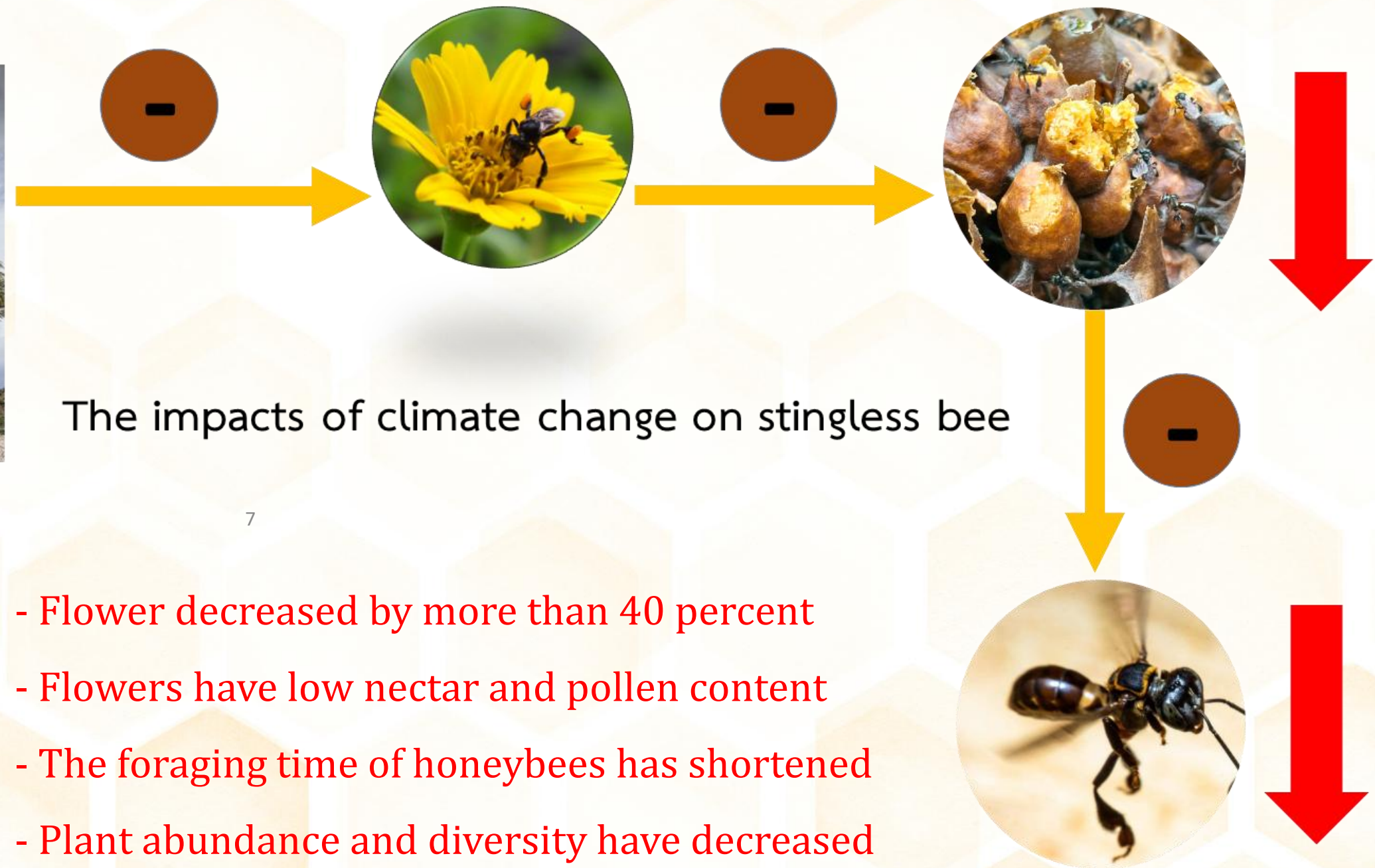
**Commercial stingless bees
in Thailand**



Necessity



Climate change



- Flower decreased by more than 40 percent
- Flowers have low nectar and pollen content
- The foraging time of honeybees has shortened
- Plant abundance and diversity have decreased
- Flowers bloom earlier than usual

Solutions for beekeepers

Honey or bee
supplementary diet



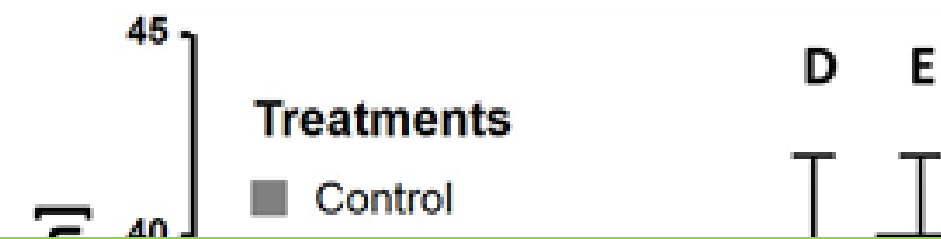
Ripe fruits such as bananas.



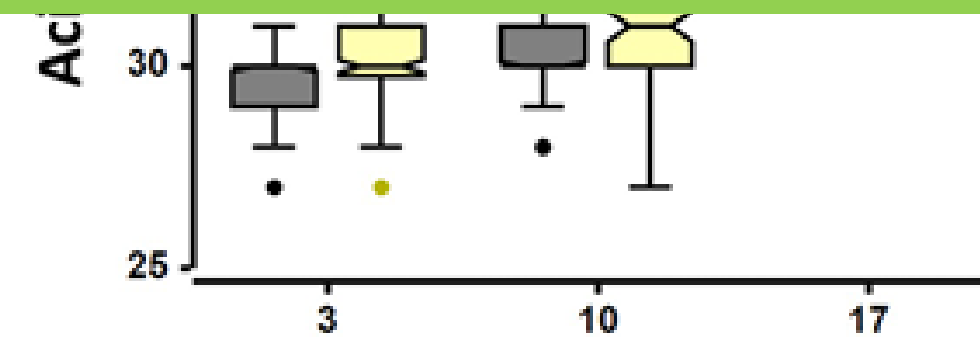
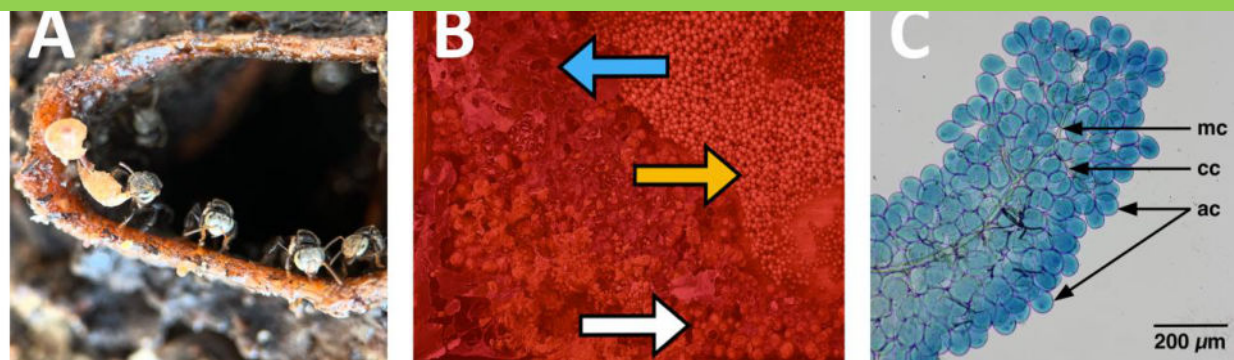
Energy drink



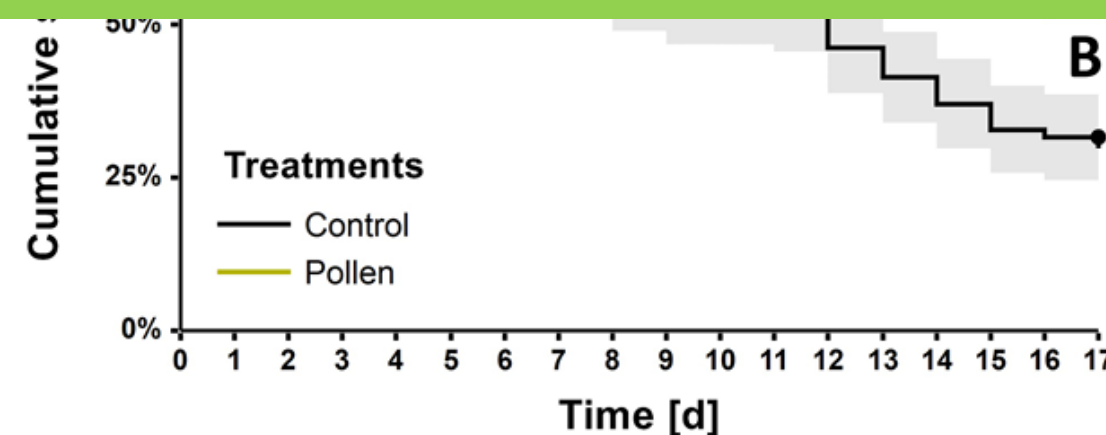
Age-dependent hypopharyngeal gland size and protein content of stingless bee workers,

A

In stingless bees, pollen affects the size of the acini.



A diet with higher protein content helps increase the survival rate.



The width of the acini increased significantly with age. (glm; $P < 0.05$)
 Pollen had a significant positive effect on acini size.(glm; $P < 0.05$)

Aim

To investigate the effects of supplementary food on the growth of stingless bees (*T. pagdeni*) both under laboratory and fields conditions.

Materials & Methods

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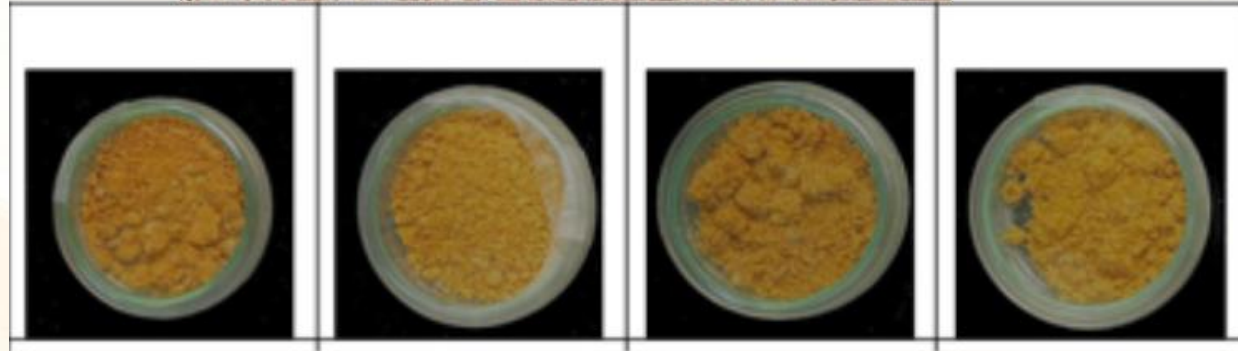
Materials and Methods



**Stingless bee (*T. pagdeni*)
70 colonies**

Supplementary diet for stingless bees

Diet	Main ingredients
Control	Bee pollen
A1	Commercial bee supplementary diet
A2	Bee pollen, Soybean meal, Probiotics
¹² A3	Bee pollen, Soybean meal, Protein extract from soybean, Probiotics



Nutritional value of artificial diets

	Commercial (A1)	A2	A3
Carbohydrate (g/100g)	32.31	14.06	13.90
Protein (g/100g)	25.27	41.22	48.71
Fat (g/100g)	3.24	3.23	2.69
Moisture (g/100g)	37.11	38.25	33.82
Ash (g/100g)	3.07	3.24	3.88
Energy (kcal/100g)	250.48	250.19	262.65

Materials and Methods

Evaluation of the effectiveness of supplementary food on the growth of stingless bee under laboratory conditions.



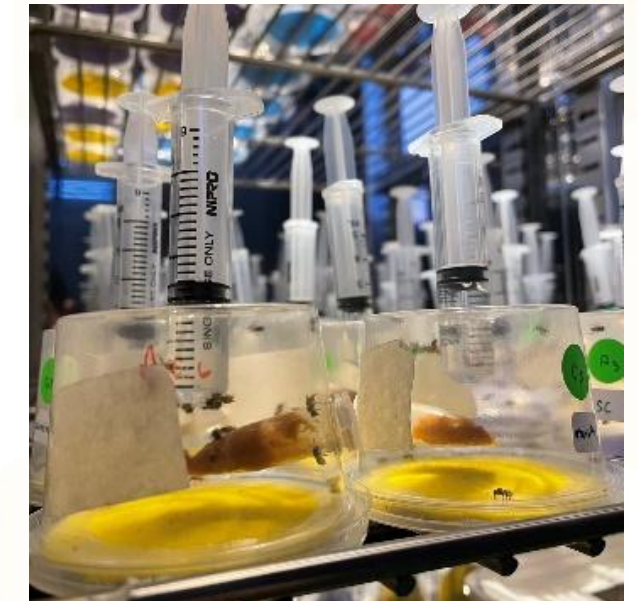
Queen right colony



Ready to emerge broods from
different 5 colonies¹⁴



Incubate at
27°C RH 70%



Newly emerge
(50 bees/cage)
(5 cages/treatment)

- A 50% sucrose solution was provided ad libitum in all experiments.
- Each supplementary diet was provided per experiment (Control, A1, A2, and A3).
- The supplementary food and sucrose solution were replaced every 3 days.

Total: 25 cages / 1,250 bees

Materials and Methods

Measurement of the size of the hypopharyngeal gland.

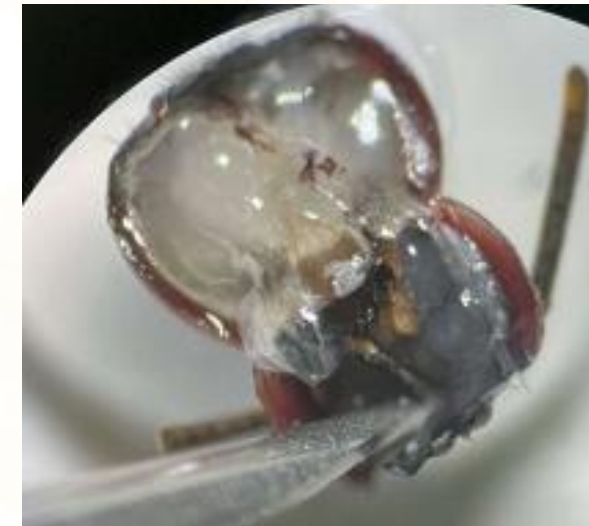


The heads of at least five stingless bees were dissected per experiment.

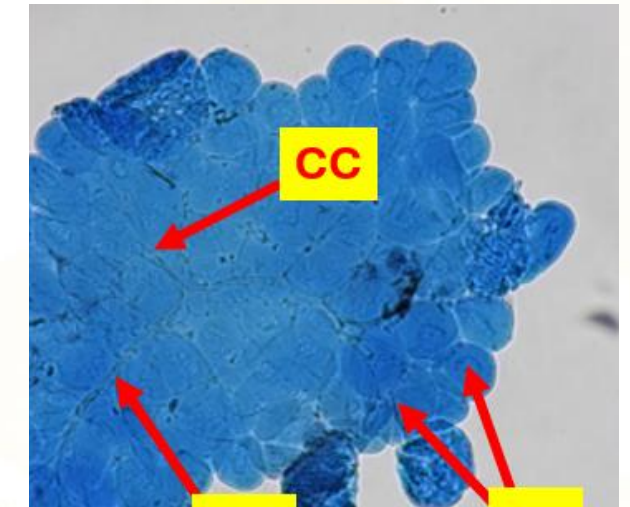


15

A scalpel was used to open both compound eyes.



The HPGs were extracted and immersed in 25 μ l of Bradford solution for 10 minutes.



The samples were observed under a microscope at 100 $\times$ magnification, and the sizes of at least 30 acini were measured per bee

Materials and Methods

Measurement of fat body content of stingless bees.



The abdomens of at least five stingless bees were dissected per experiment.



**Dry at 70 °c
for 48 hours**



The samples were left to cool in a desiccator and then weighed.



**Soak in 1.5 ml
Diethyl ether
for 24 hours, dried and
weight**

Materials and Methods

Evaluation of the effectiveness of supplementary food on stingless bee under field conditions.

Experimental area



Samut Prakan
13.67003, 100.57180



Khon Kaen
16.49619, 102.88493



Rayong
12.70244, 101.24131

Materials and Methods

Evaluation of the effectiveness of supplementary food on stingless bee under field conditions.

Area	With Diet	Without Diet
Rayong	10	10
Samut Prakan	10	10
Khon Kaen	10	10



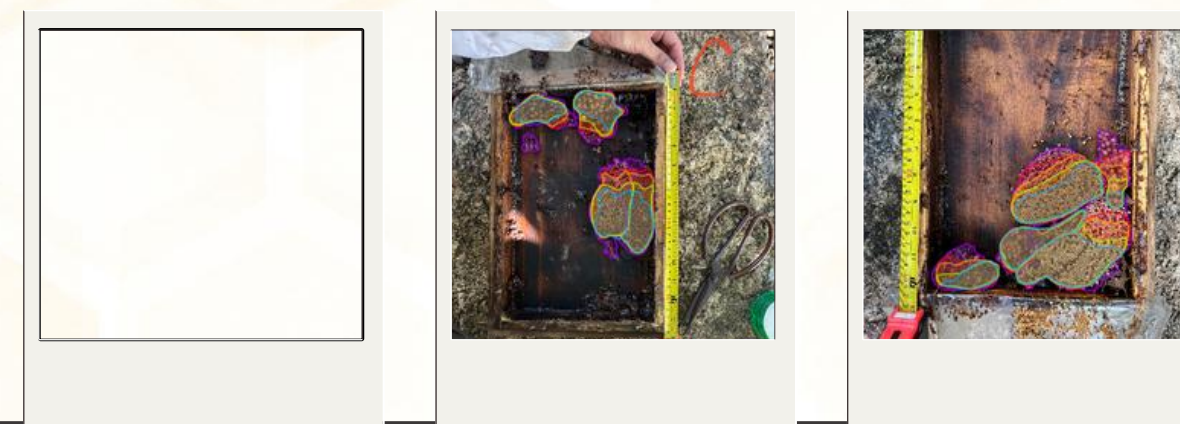
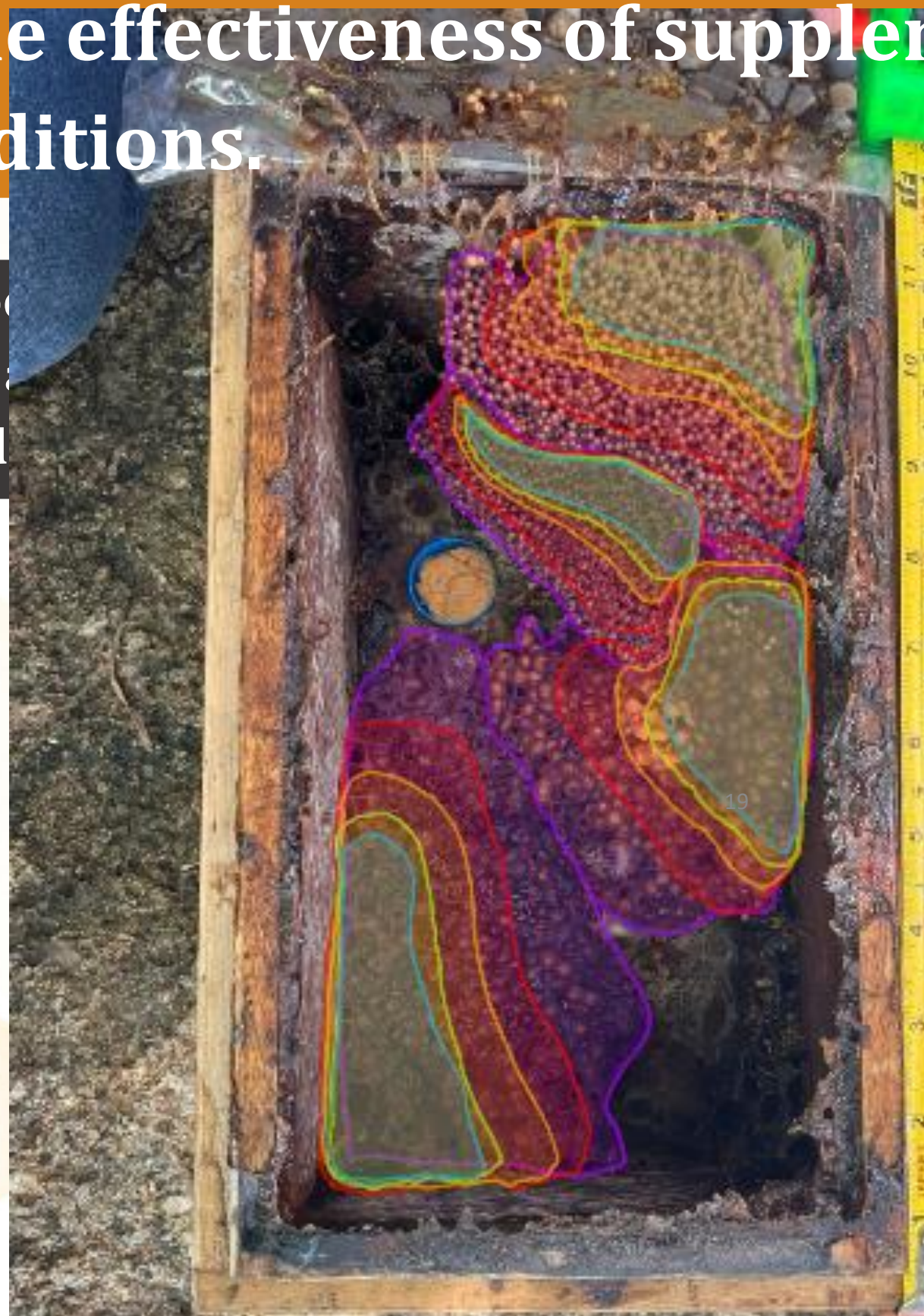
Feed with diet 3g/colony/week for 1 month

Packed into small, sealed foil bags, each containing 10 grams, to facilitate use by beekeepers.

Materials and Methods

Evaluation of the effectiveness of supplementary food on stingless bee under field conditions.

Supplementary food
the stingless bees
7 d



Take photographs of the internal structures of the colony every 7 days for at least 1 month.



Analyze the changes in the internal structures of the colony before and after supplementary feeding, and compare them with the control group using ImageJ.

Materials and Methods

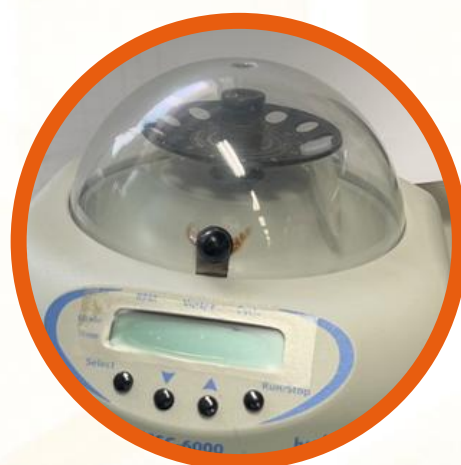
The trehalulose content in the honey samples using LC-Q-TOF-MS



Honey 2 g
+ DI water 1 ml
+ Acetonitrile 4 ml



Vortex
for 5 min



Centrifuge at
2000 rpm for 3 min



Filtered by syringe filter
(PTFE) 0.2 μ m



Analyzed by High Resolution LC-MS/MS QTOF

สภาวะที่ใช้ในเครื่อง (UHPLC condition)

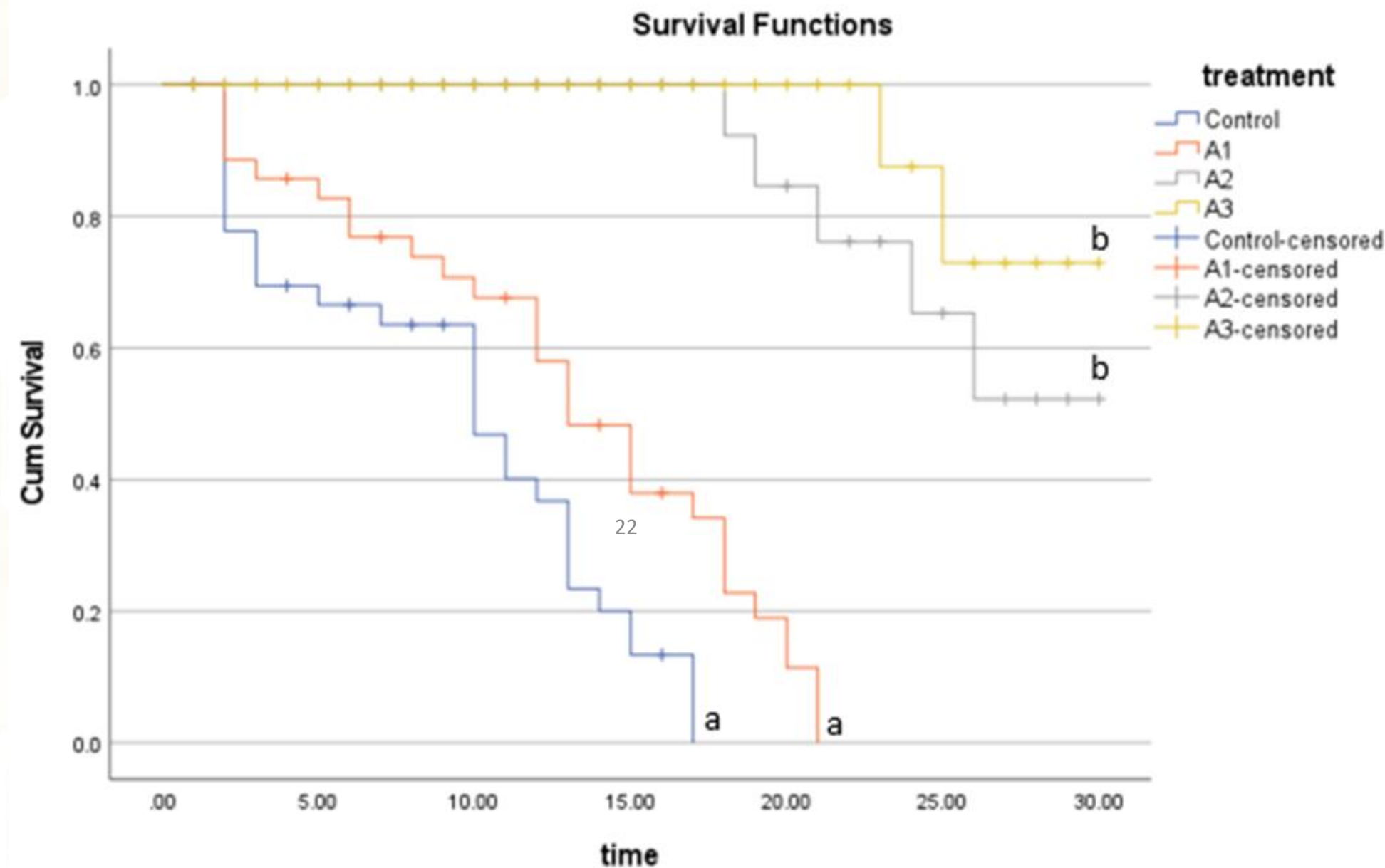
UHPLC	Agilent 1260 infinity II
Column	Poroshell 120 HILIC-Z 2.1*100 mm 2.7 μ m,Agilent,USA
Column Temperature	35 $^{\circ}$ C
Flow rate	0.2 ml/min
Injection volume	2 μ l
Mobile phases	A : 30% ACN/70% water (++0.1%NH ₄ OH) B : 80% ACN/20% water (++0.1%NH ₄ OH)

Results & Discussion

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RESULTS

The effect of supplementary food on the survival rate of stingless bee *Tetragonular pagdeni* under laboratory conditions.

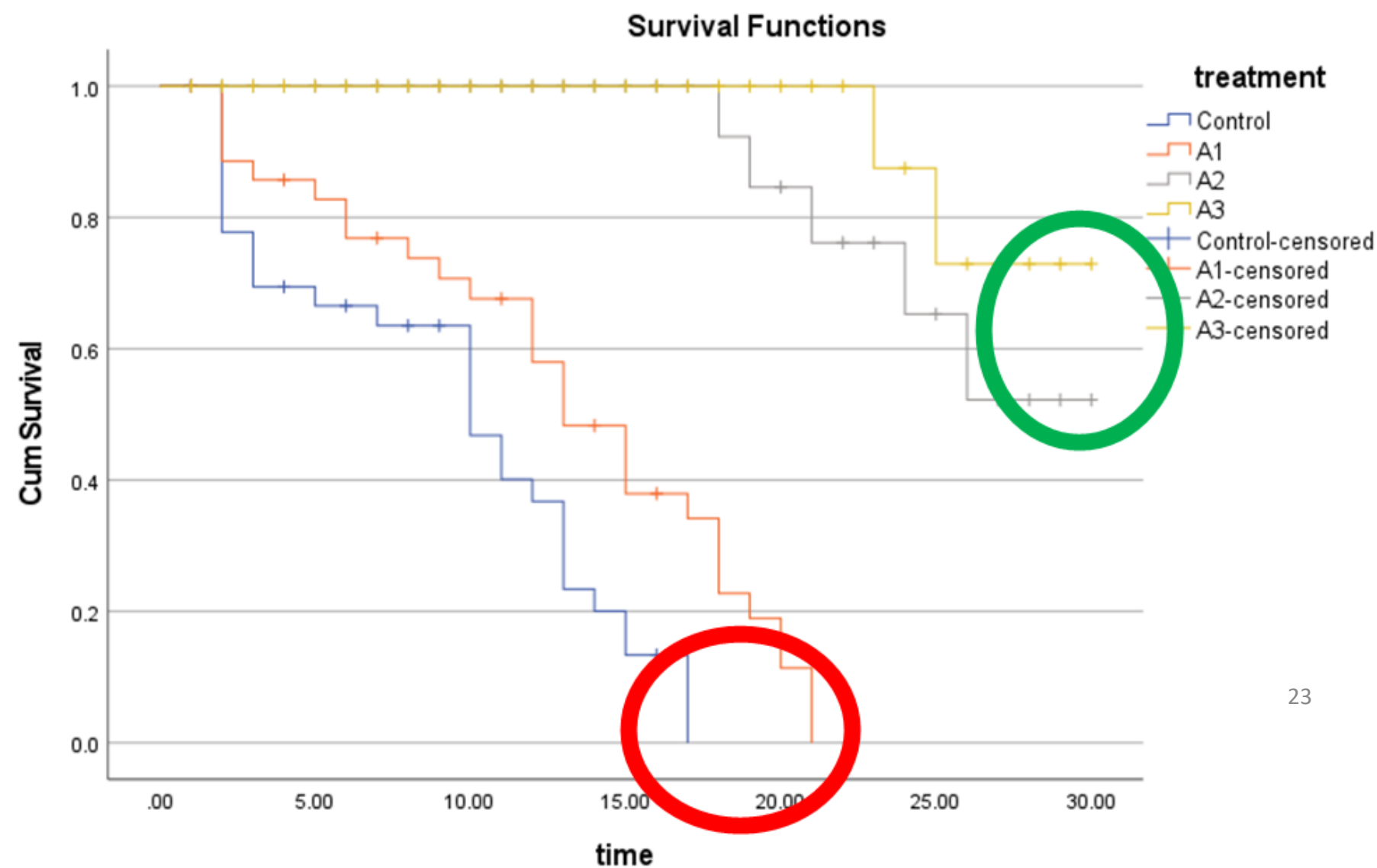


- The control group and treatment A1 stingless bees were unable to survive until the end of the experiment.

- A2 and A3 stingless bees had survival rates of 56.67% and 67.78%, respectively.

Figure 1 Curve of survival rate when reared in cage under laboratory conditions ($27 \pm 2^\circ\text{C}$ and 70 %RH) for 30 days. The superscript letters are significantly different ($p < 0.05$).

DISCUSSION AND CONCLUSIONS



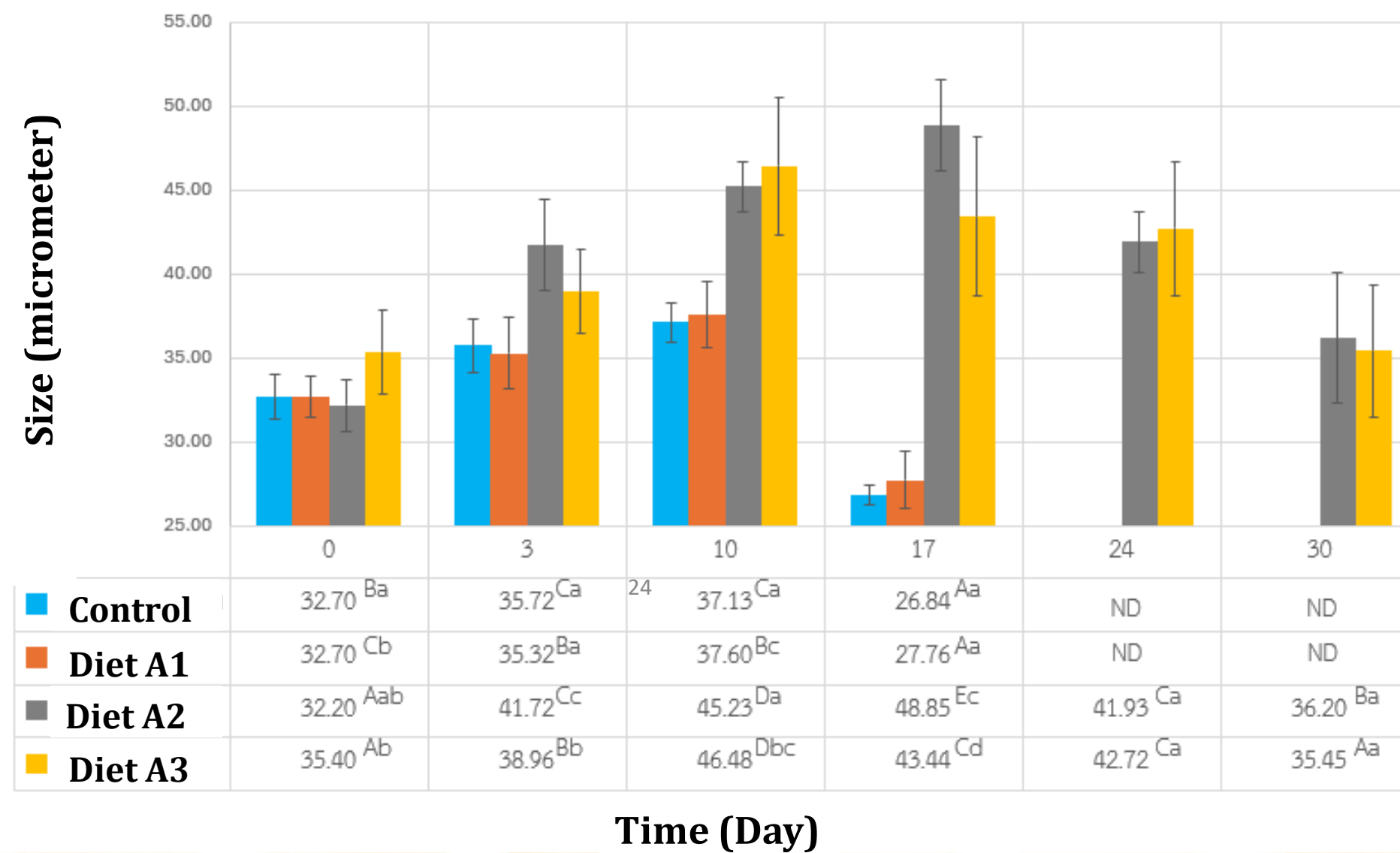
23

From the graph, it can be seen that...

- The control group and A1, stingless bees were **unable** to survive until the end of the experiment.
- The control and A1 group, which represent general bee pollen and commercial bee food commonly provided by beekeepers in stingless bee colonies, did not support the survival of stingless bees during periods of food scarcity.
- High-protein diets enhanced survival rates, which is consistent with the findings of **Straub et al., 2024**.

RESULTS

The size of the hypopharyngeal gland



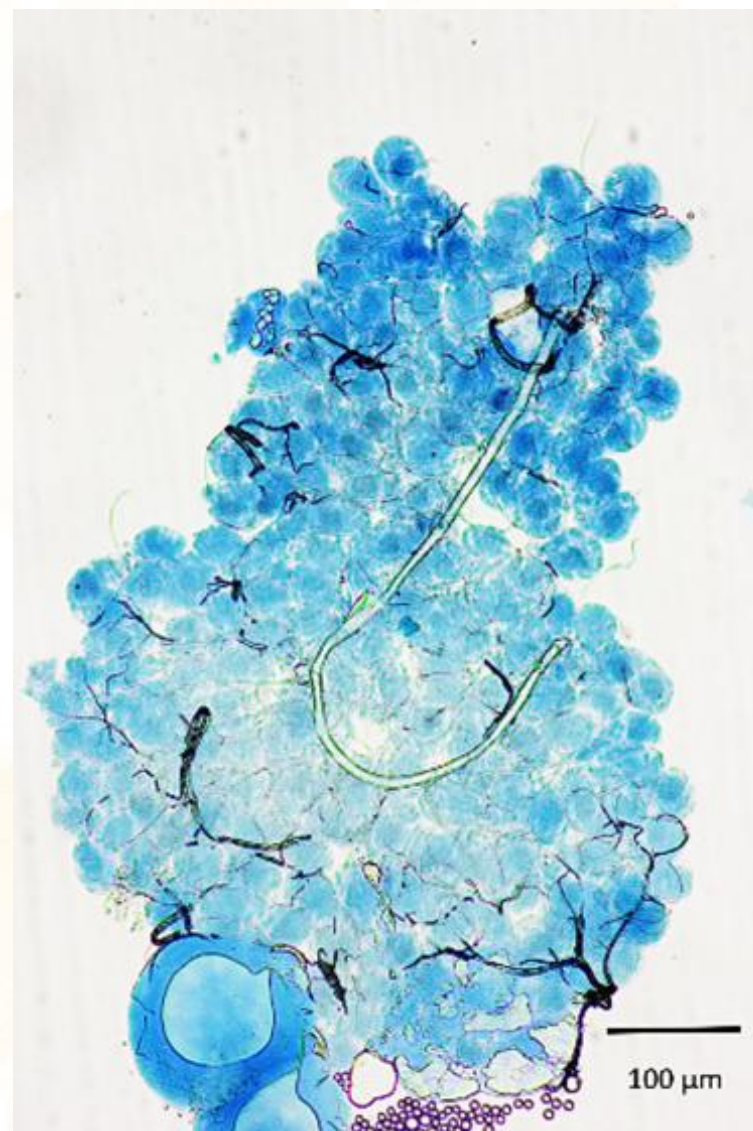
On day 30, diets A2 and A3 resulted in HPG sizes of $36.20 \pm 3.90 \mu\text{m}$ and $35.45 \pm 3.90 \mu\text{m}$, respectively, with no significant difference between them.

Figure 2 Curve of hypopharyngeal gland size when reared in cage under laboratory conditions ($25 \pm 2^\circ\text{C}$ and 70 %RH) at 0, 3, 10, 17 days).

RESULTS

The morphology of the hypopharyngeal gland

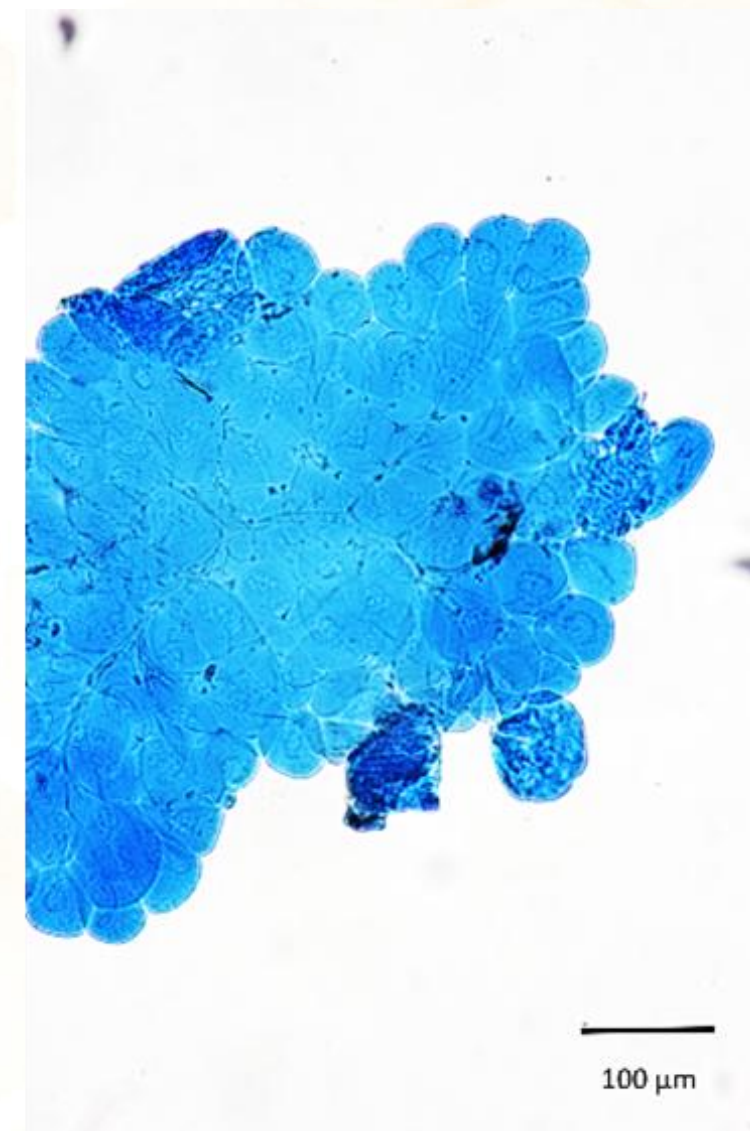
HPGs มีความสมบูรณ์มากกว่า



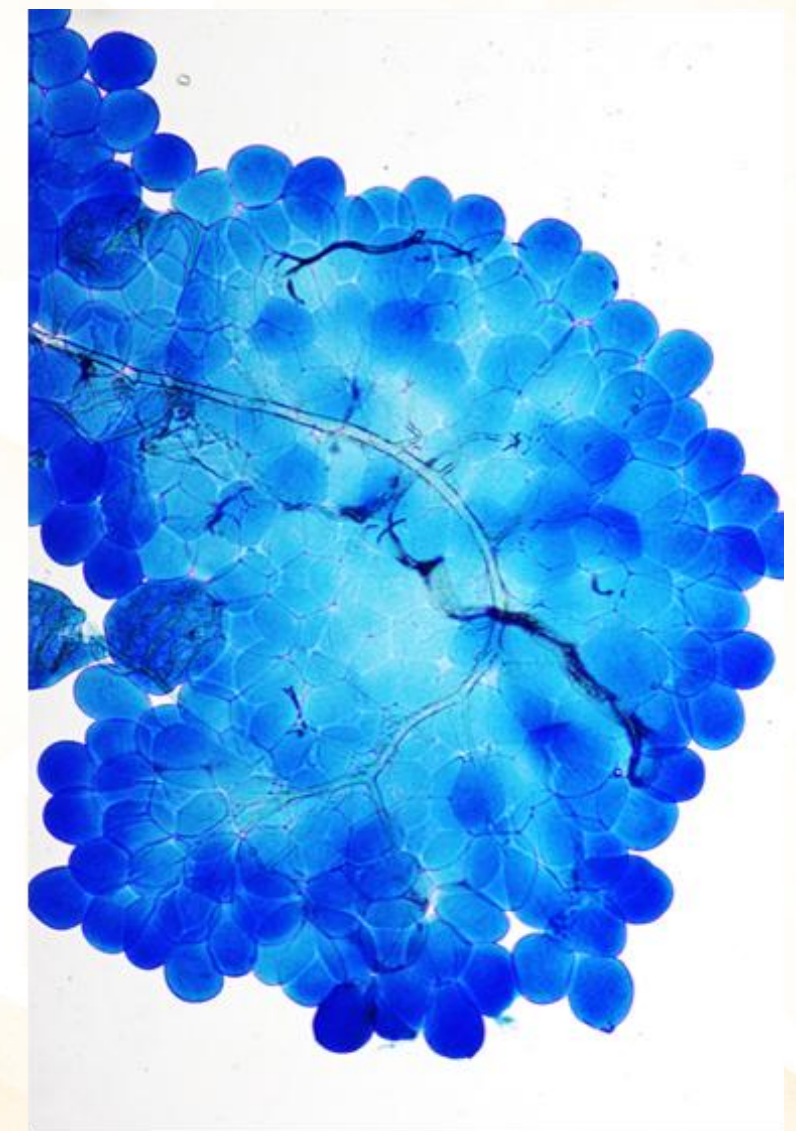
Control



A1



A2

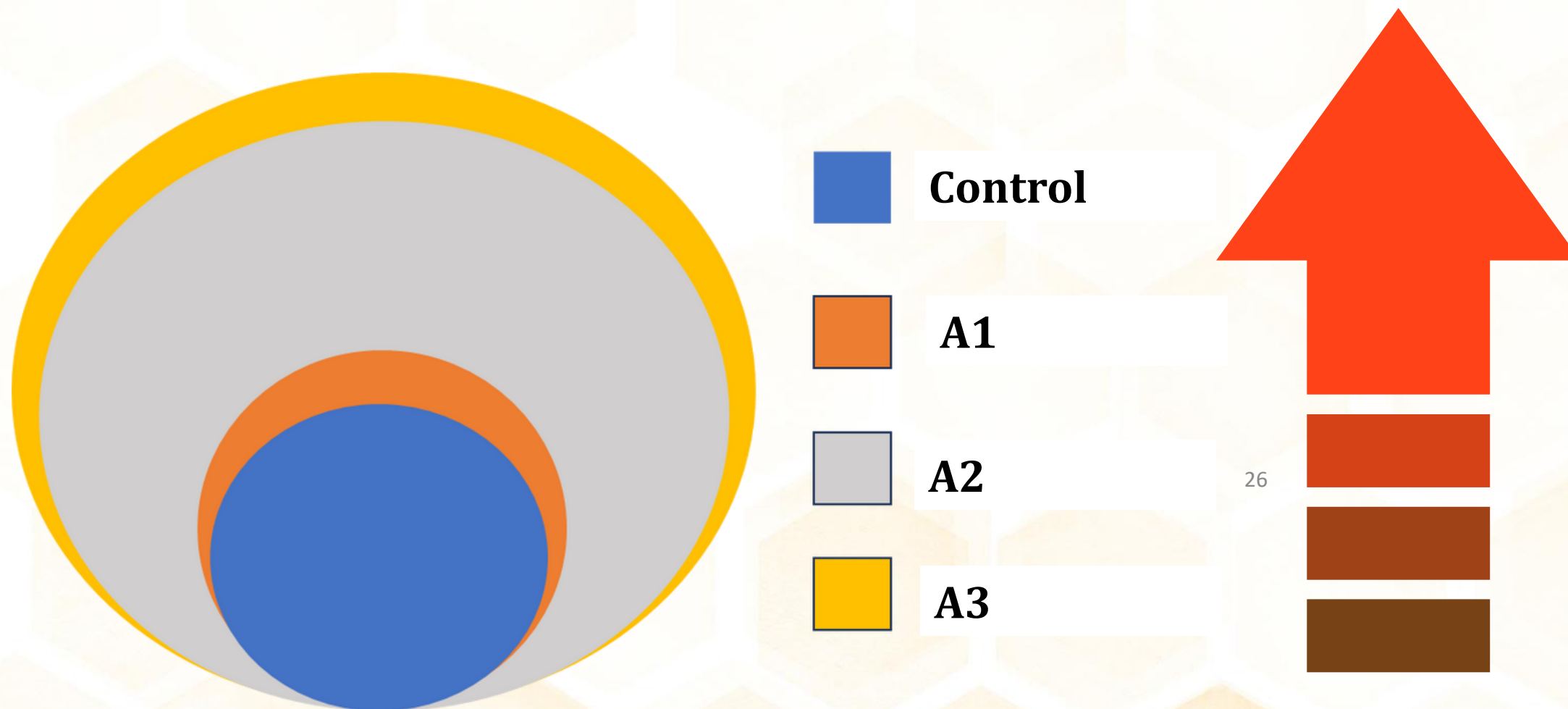


A3

Figure 3 Characteristics of the hypopharyngeal gland of stingless bee (*T. pagdeni*) fed various dietary supplements for ten days under a microscope at 100x magnification.

DISCUSSION

Hypopharyngeal gland size



**Increased by up to
1.85 times.**

- The larger size of the HPGs is likely due to the protein content, which is consistent with the findings of Straub et al., 2024 and Vanessa et.al, 2019.

RESULTS

The fat body contain

The body fat content of stingless bees did not decrease but rather increased. On day 30, bees fed with diets A2 and A3 had fat contents of **0.18 ± 0.00 mg/bee** and **0.24 ± 0.00 mg/bee**, respectively.

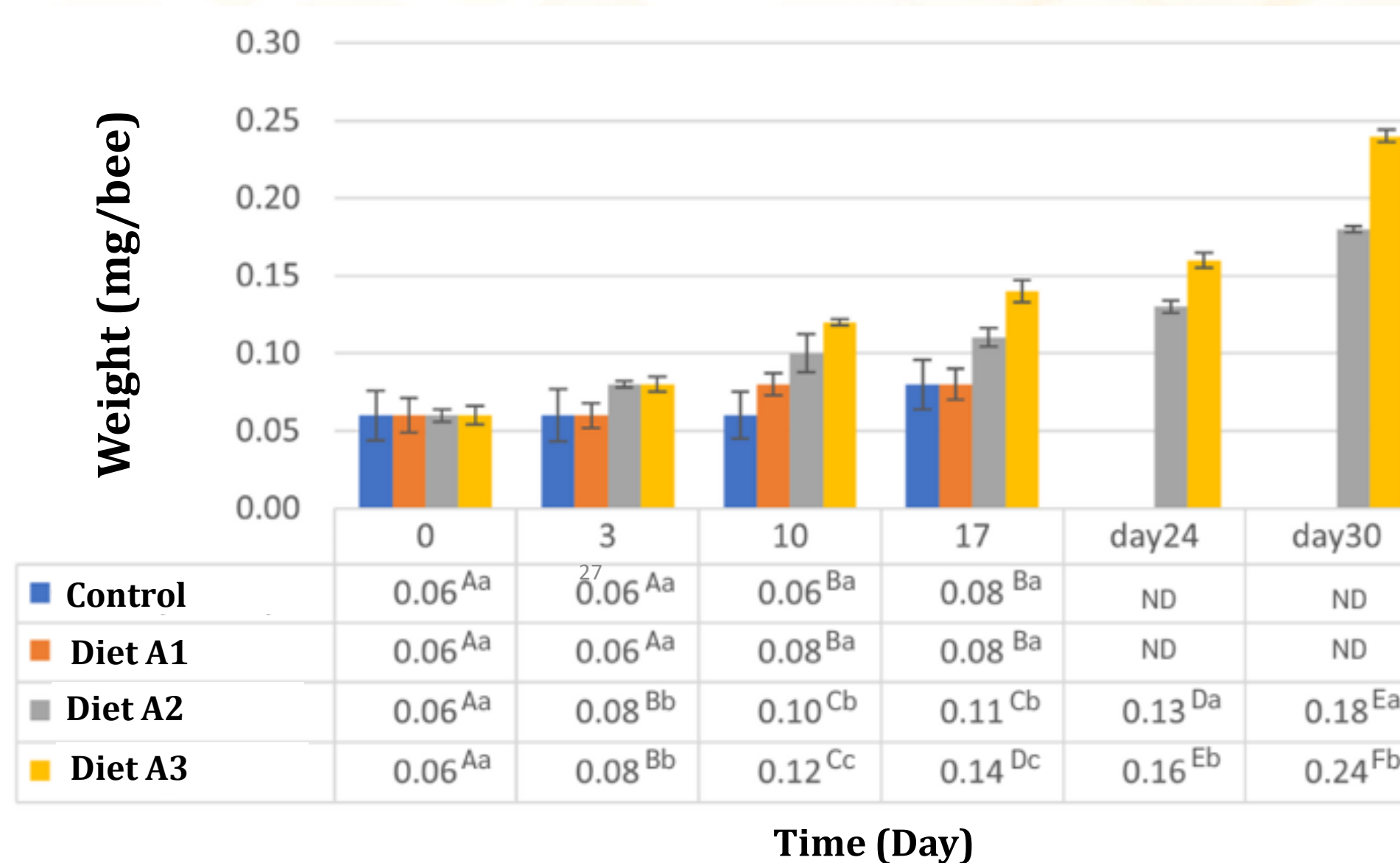


Figure 4 Curve of the fat body when reared in cage under laboratory conditions (25 ± 2 °C and 70 %RH) at 0, 3, 10 and 17 days. N.D.=not detect.

DISCUSSION

- **The increase in body fat content enables stingless bees to store more food and enhances their ability to detoxify substances such as pesticides.**
- **Based on the study by Vanessa et al., 2019, it can be concluded that stingless bees receiving supplementary food were adequately nourished.**
- **This indicates that the supplementary diets A2 and A3 in the present study can effectively serve as substitutes for natural food sources.**

RESULTS

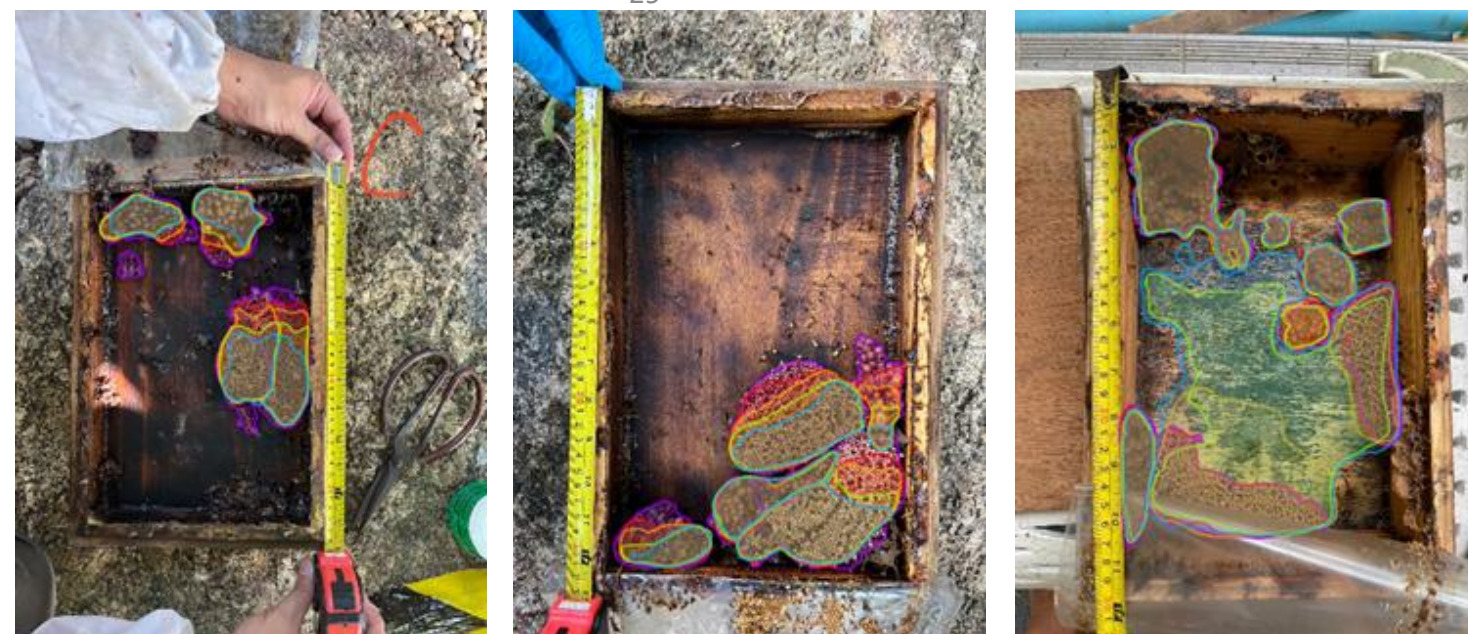
Field test results on the effectiveness of supplementary food on the growth of stingless bee without supplementary feeding.

Day 0



Blue = Day 0
Green = Day 7
Orange = Day 14
Red = Day 21
Purple = Day 28

Day 30



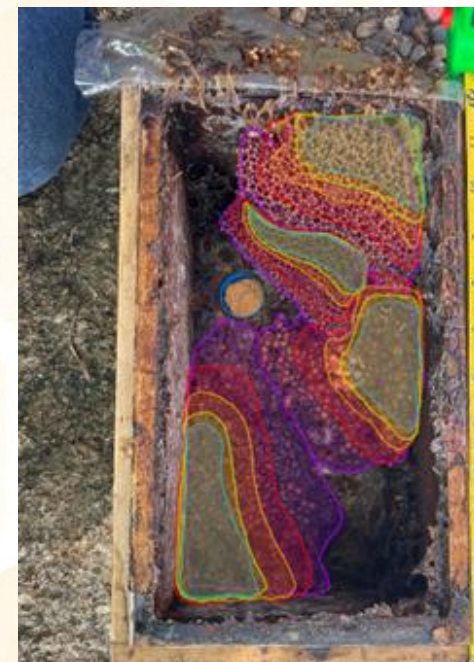
RESULTS

Field test results on the effectiveness of supplementary food on the growth of stingless bee that received supplementary feeding.

Day 0



Day 30



Blue = Day 0
Green = Day 7
Orange = Day 14
Red = Day 21
Purple = Day 28

RESULTS

Field test results on the effectiveness of supplementary food on the growth of stingless bees.

	Young Broods (CM ²)	Mature Broods (CM ²)	Pollen pots (CM ²)	Honey pots (CM ²)	Honey (ml)
Control (Rayong)	↑ 17.74±0.57	↑ 22.94±3.99	↑ 27.83±9.85	↑ 22.09±4.84	69.74±1.85
With Supplementary diet (Rayong)	↑ 71.13±7.65	↑ 104.69±27.91	↑ 81.54±22.20	↑ 57.83±12.75	133.61±27.35
Control (Samut Prakan)	↑ 41.99±11.24	↑ 44.94±4.77	↑ 28.69±4.90	↑ 28.51±7.24	48.86±3.53
With Supplementary diet (Samut Prakan)	↑ 69.72±10.39	↑ 82.65±14.07	↑ 50.48±15.99	↑ 51.90±4.22	136.57± 5.59

DISCUSSION

- **Supplementary feeding had a significant effect on the development of internal nest structures, stimulating stingless bees to build more internal components compared to colonies that did not receive supplementary food.**
- **Colonies that received supplementary feeding were able to produce 1.96 to 2.73 times more honey.**
- **In addition, supplementary feeding helps reduce the risk of colony collapse that may result from food scarcity.**

RESULTS

The trehalulose content in the honey samples using LC-Q-TOF-MS

Sample	Trehalulose contains (ug/g)
RY-C	59.83

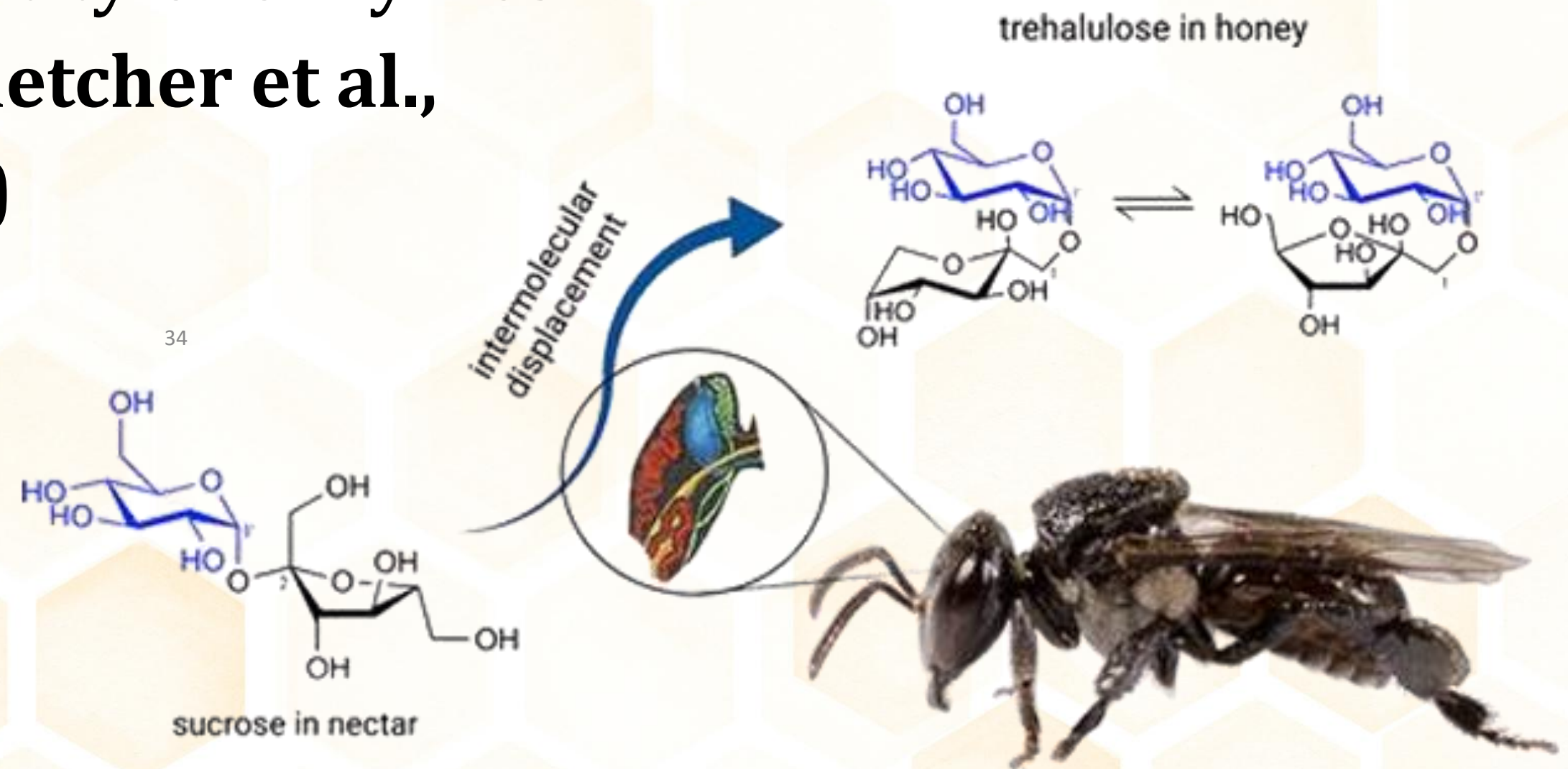
Trehalulose is found only in stingless bee honey and is absent in honey from *Apis mellifera* or *Apis cerana*.

Moreover, stingless bee honey from colonies receiving supplementary feeding showed an increase in trehalulose content—up to more than **twofold**—across all research sites.

<i>Apis mellifera</i>	Not detected
<i>Apis cerana</i>	Not detected

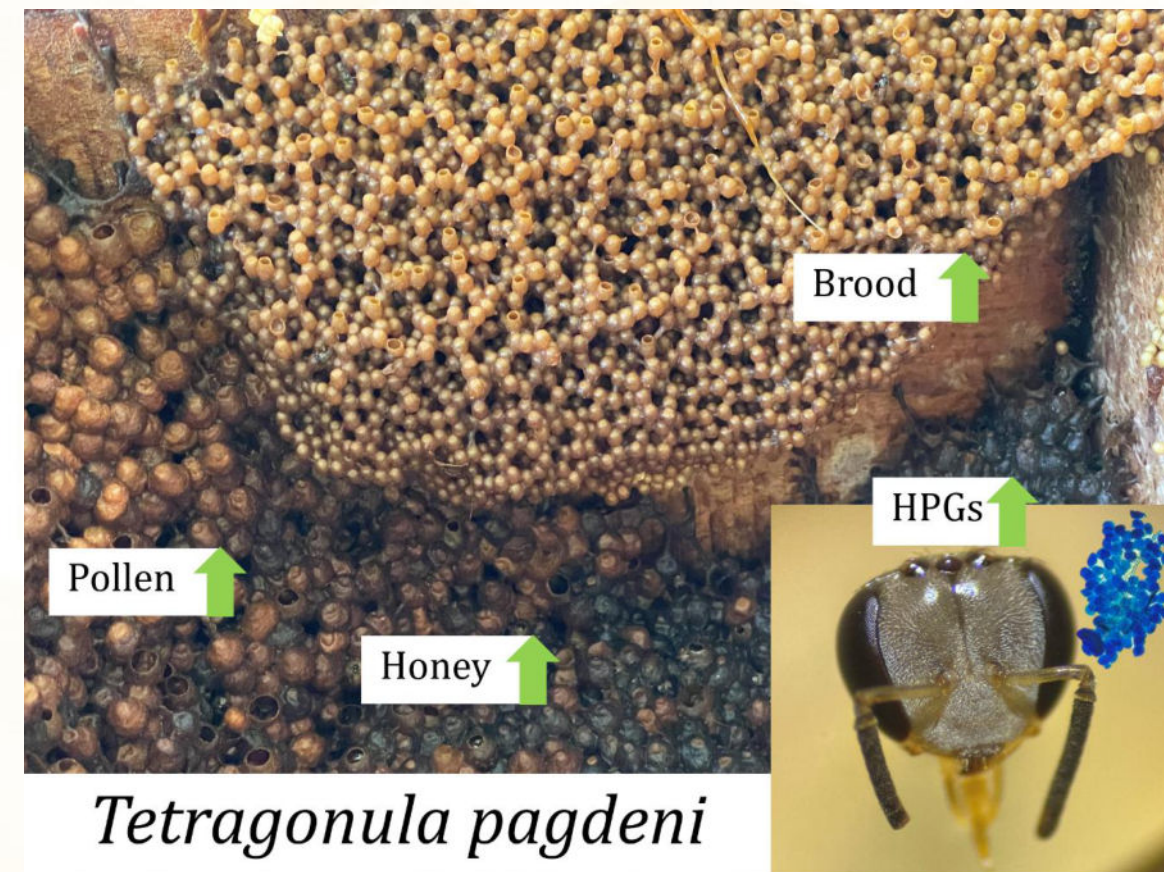
DISCUSSION

- The increased size of the HPGs affects the amount of trehalulose, as trehalulose levels depend on the quantity of enzymes produced by the HPGs. (Fletcher et al., 2020; 8 Zhang et al., 2022)



Conclusion

- To our knowledge, this is the first study to utilize the stingless bee, *Tetragonula pagdeni*, to demonstrate the importance of a supplementary diet for the survival of stingless bees, as well as for HPG acini width and fat body in laboratory conditions, and colony survival, population, and honey production in colony conditions.



Conclusion

- Our results highlight the importance of supplementary diets and nutrition for stingless bees to promote individual healthiness and to reduce their susceptibility to food shortages.





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Thank You

For Your Attention

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