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ORIGINAL RESEARCH ARTICLE



# Optimum sugar syrup feeding intervals for *Apis cerana* Fab. during the winter dearth period in the sub-tropical area of Nepal

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# **ARTICLE HISTORY**

Received: 28 September 2024 Revised received: 18 November 2024 Accepted: 28 November 2024

- Keywords
- Apis cerana Artificial feeding Feeding interval Sugar syrup Winter dearth

# ABSTRACT

The study aimed to identify the optimal sugar syrup feeding interval for honeybee colonies during the winter dearth period in subtropical region of Nepal to ensure colony survival and maintenance. The focus was on evaluating sealed brood area, sealed honey area, egg-laying activity, and pollen collection. The experiment was conducted in an apiary located in Lamjung district of Nepal. Sugar syrup (1 water: 1 sugar) was fed to honeybee colonies at intervals of 7, 10, 15, and 20-days, along with a control group. Each treatment was replicated across four replications with five beehives in each replication, and data were collected weekly. Parameters such as the sealed brood area, honey storage, egg-laying rate, and pollen cells were measured by counting eggs, pollen cells, and assessing areas covered by sealed brood and honey. Colonies fed sugar syrup at 15-days intervals exhibited the best performance across all measured parameters, including the largest sealed brood area, highest honey reserves, maximum eggs laid by the queen bee, and the most pollen cells. These results indicate that feeding at 15days intervals supports optimal colony health and activity during low-temperature winter conditions. Feeding sugar syrup at 15-days intervals is the most effective practice for maintaining colony health, ensuring optimal honey reserves, brood production, and pollen collection during the winter dearth period in subtropical areas of Nepal. This feeding strategy is recommended for improving colony survival, maintenance, and overall performance during challenging winter conditions in subtropical region of Nepal.

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**Citation of this article:** Khanal, D., Aryal, S., Bista, S., Kafle, K., Bastakoti, B., & Banjade, D. (2024). Optimum sugar syrup feeding intervals for Apis cerana Fab. during the winter dearth period in the sub-tropical area of Nepal. *Archives of Agriculture and Environmental Science*, 9(4), 755-760, https://dx.doi.org/10.26832/24566632.2024.0904017

# INTRODUCTION

Apis cerana, often recognized as the Asiatic honeybee (or Eastern honeybee), are little honeybees that are native to southern and southeast Asia, including all Himalayan countries. Apis cerana can be found at altitudes ranging from sea level to 3,500 meters in places with suitable flora and environment (Ji *et al.*, 2023). This bee species has evolved to harsh environmental circumstances and can withstand high-temperature variations as well as prolonged periods of rainfall (Alburaki *et al.*, 2023). A. *cerena* can tolerate temperatures as low as -0.1 C, which is deadly to other bee species. *A. cerana* is one of Nepal's four native honeybees (Aryal *et al.*, 2016). In case of Nepal, organic farming and beekeeping are tightly linked to each other where *A. cerena* has essential role as a pollinator in farmer's field (Bastakoti & Khanal, 2022). Traditionally, colonies are housed in logs, walls, or hives, each producing 4.5 kg of honey on average and *A. laboriosa*, native bee species of Nepal prepare open nesting in a single comb in the cliffs (Banjade *et al.*, 2024.). Beekeepers typically harvest honey twice a year from traditional hives, yielding 9 kg of honey per hive yearly. Three subspecies of A. cerana have been discovered in Nepal: *A. cerana cerana*, *A. cerana*  *himalaya*, and *A. cerana indica*. Bees are also important pollinators, pollinating mountain crops, particularly early flowering fruits and vegetables. The amount and quality of pollen and nectar arriving in the hive and the hive's food reserves determines whether bees want additional food. Compared to unfed colonies, supplemental feeding with a mixture of sugar, pollen, and soybean flour may boost brood growth by 81 percent (Amera *et al.*, 2024).

In comparison, sugar as a nectar replacement alone may increase brood-rearing by 40 percent during the dearth period (Ahmad *et al.*, 2021). Colonies with sufficient food storage in the autumn may only require additional meals in the spring if the winter is exceptionally lengthy (Brodschneider & Crailsheim, 2010). However, extra meals and ongoing brood-rearing may be necessary before and after the flowers open if the weather is abnormally cold and wet. The lack of best sugar syrup feeding practices among the bee apiary farmers has resulted in dead bee hives during the extreme winter period and has caused financial loss. The study aims to identify the best sugar syrup feeding interval for Apis cerena in the subtropical region of Nepal.

# MATERIALS AND METHODS

# Description of the study area

The preliminary information on beekeeping and resource availability was gathered at Sundarbazar municipality's ward no. 5 in Gahate village in the Lamjung District. It was discovered that the farmers of this village are rearing their bee hives despite the lack of bee flora during the winter season.

#### Period, location, and climate

The experiment was conducted during the winter dearth period in the farmer's apiary in Sundarbazar-5, Gahate Lamjung, Nepal. Lamjung district lies on the geographical coordinates of 28.1634° N, 84.4050° E, and altitudes of 1050 meter above sea level (masl). Lamjung district is part of the Western Development Region of Nepal. The experiment was conducted on Mr. Dil Bahadur Gurung's apiculture farm.

# **Experimental setup**

Twenty *Apis cerena* colonies were selected from the apiary for the experiment. To mimic natural conditions, all colonies were equalized a week prior to the start of the experiment, ensuring a similar bee population across the hives. The study employed a randomized complete block design (RCBD), with each treatment replicated four times. The colonies were fed a 50% sugar solution (1:1 sugar to water) as an artificial diet. Using a frame feeder, each colony received 100 ml of this sugar solution. To ensure consistency, all treatments were applied during the evening between 5 pm and 7 pm for the duration of the experiment. Additionally, weekly cleaning of the hives was carried out to prevent ant infestations and other disturbances. The standard methodology for this experiment was developed by following (Zhang *et al.*, 2019) protocol with some modifications.

# Preparation of feeding material (Sugar syrup)

Sugar syrup was prepared by dissolving an equal volume of crystal sugar with an equal volume of freshwater, i.e., 1:1. Crystal sugar was poured in boiled water and then stirred till crystal sugar was completely dissolved.

# Data recording and statistical analysis

The area covered by sealed brood cells (pupa cells) and sealed honey cells (cells with ripened honey), the pollen cells, and the egg-laying by the queen bee were recorded at weekly intervals. The data from the tested parameters were statistically analyzed to determine the differences between treatments. The bar graph, general trend graph, and linear regression analysis of various parameters were computed using Microsoft Excel 16.

Table 1. Temperature data of the research location during the research period.

Date	Weekly average maximum Temperature.	Weekly average minimum Temperature
23 December to 29 December	13.51286(±0.713078)	6.642857(±0.877924)
30 December to 5 January	15.61(±0.95347)	7.36(±0.400724)
6 January to 12 January	12.50571(±0.488052)	8.88(±0.416315)
13 January to 19 January	15.39571(±1.279946)	9.087143(±0.587981)
20 January to 26 January	16.44143(±0.394422)	6.691428(±0.428578)
27 January to 2 February	19.12714(±0.357809)	8.42(±0.230996)

Source: Meteorological data for research period, GRS Bandipur, Nepal.

Table 2. Different treatments applied to the experiment.

Treatment	Description
T1	Control-without artificial diet
T2	Sugar syrup feeding at weekly intervals
ТЗ	Sugar syrup feeding at ten-day intervals
T4	Sugar syrup feeding at fifteen-day intervals
T5	Sugar syrup feeding at twenty-day intervals

# **RESULTS AND DISCUSSION**

#### Sealed brood area

All treatments, except the 10-days feeding intervals, showed an increased sealed brood area at the end of the experiment compared to before. 15-days of feeding intervals treatment has increased the total sealed brood area by 5.29 times, which is the highest, followed by control treatment, which has increased the average sealed brood area by 3.13 times, while 10-days of feeding intervals treatment has decreased the total sealed brood area by 0.64 times (Zhang et al., 2019) reported the similar findings. The observed data recommends that to maintain the sealed brood area in the case of A. cerena during the winter dearth period of the subtropical region of Nepal, a 15-days feeding intervals is the best practice, which not only maintains but also can increase the sealed brood area in winter dearth period. Each treatment showed a unique response to the increase in the sealed brood area for each week of the experiment. The highest slope of the linear regression line is obtained for control, which is 16.904 (R<sup>2</sup>=0.62), followed by 15-days of feeding intervals with a slope of the linear regression line 12.954 (R<sup>2</sup>=0.62). Among all, only 10-days of feeding intervals has a negative slope of the linear regression line, i.e., 2.5464 ( $R^2$ =0.27). The results showed that the control group had the highest rate of increase in the sealed brood area, followed by 15-days intervals during the research period. (Gemeda et al., 2018) reported similar findings on A. mellifera in Nigeria and found that the growing population numbers of honeybees given sugar syrup remained somewhat lower than those not fed sugar syrup over the first five months of monitoring. Because honeybees did not readily take the syrup initially, as predicted, due to an abundance of nectar and pollen available from wild flora and crops at the time. The control treatment shows the highest slope for the linear regression line, possibly due to its higher initial average brood area before the experiment. At the end of the experiment, 15-days feeding intervals increased the sealed brood area by a greater amount, supported by (Peirson et al., 2024), who documented that supplemental sugar feeding can assist the colony to survive or maintain brood raising and colony expansion. The findings of this study do not align with (Prakash et al., 2014), who identified that one-week intervals have the most increased brood area, followed by twoweeks intervals, three weeks intervals have very little and negligible brood rearing, and four-weeks intervals have almost no brood area, which may be due to different bee species, sugar concentration and feeding methods.

#### Sealed honey area

For the sealed honey area, all treatments showed a decreased total sealed honey area at the end of the experiment compared to before the experiment. However, the reduction rate in the sealed honey area is different for different treatments. Among all treatments, the 20-days feeding intervals has decreased the sealed honey area by a greater extent, i.e., by 0.63 times. Still, the 7-days and 15-days feeding intervals have shown a minimum reduction of the sealed honey area. 7-days feeding intervals showed decreased sealed honey area only by 0.44

times, followed by 15-days feeding intervals, i.e., 0.49 times, which indicates that 7-days feeding intervals can check the faster rate of reduced sealed honey area and maintain it for the winter dearth period. Different feeding interval treatments are differentially responsible for maintaining a sealed honey area during the winter dearth period as per supplementary Figure 1. The analysis found that the slope of the linear regression line is highest for 10-days intervals, i.e., 52.47 (R<sup>2</sup>=0.92), which indicates the faster rate of reduction of the sealed honey area during the research period, while 7-days have shown the lowest slope of the linear regression line, i.e., 25.37 (R<sup>2</sup>=0.96), followed by 15-days feeding interval, i.e., 34.58 (R<sup>2</sup>=0.90). This finding can recommend that to maintain the sealed honey area in the beehives during the winter dearth period, 7-days intervals of sugar syrup feeding are the best for the subtropical region of Nepal in A. cerena. (Vijayakumari et al., 2022) suggested that supplementary feeding with sugar and water can significantly enhance colony growth and health during dearth periods, which is crucial for maintaining strong and productive bee colonies. (Naveen et al., 2024) reported similar findings on A. mellifera in Krishi Vigyan Kendra Morena district, India, where they observed that giving sugar solution to A. mellifera colonies at 7-days intervals was particularly advantageous to colony growth and honey storage throughout the dearth period. Increased honey production in sugar-fed colonies has been reported by (Sultana et al., 2024).

#### Egg laying by queen bee

The total number of eggs laying by the queen bee increased at the end of the experiment for all sugar syrup feeding intervals, including the control condition. Although all treatments have enhanced the total number of eggs, the change in the increment of the eggs at the end of the experiment is different for each specific treatment. Among tested treatments, a 15-days sugar

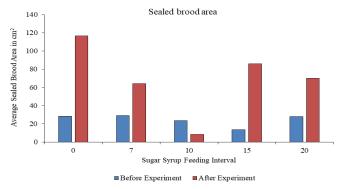
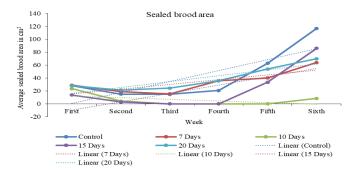
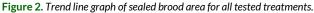


Figure 1. Effect of different feeding intervals on average sealed brood area.

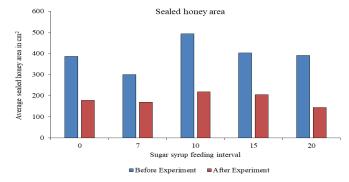


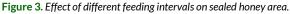


syrup feeding intervals increased the total eggs by the greatest extent, i.e., 36.70 times, followed by a 7-days feeding intervals, i.e., 14.61 times. However, the control treatment increased their total number of eggs by the least amount, i.e., only by 3.83 times. So, to expand the beehive colony even in the winter dearth period, the colony must be fed with alternative sources to natural pollen and nectar. In the case of sugar syrup feeding practices, 15 and 7-days are the best feeding intervals practices to expand the colony during the winter dearth period in the subtropical region of Nepal. The total number of eggs laying by the queen bee showed a different pattern in each week for each treatment shown in supplementary Figure 2. As per the trend lines graph, each treatment differentially regulates the total number of eggs laid by queen bees. Among all treatments, 7days of feeding intervals has the highest slope for the linear regression line, i.e., 77.871 (R<sup>2</sup>=0.8487), followed by 15-days of feeding intervals, i.e., 95.40 (R<sup>2</sup>=0.67). The lowest slope of the linear regression line is observed for 10-day feeding intervals, i.e., 50.05 (R<sup>2</sup>=0.45), followed by control treatment, i.e., 69.471 (R<sup>2</sup>=0.87). The results revealed that 7-days intervals feeding resulted in the highest incremental egg-laying, followed by 15days intervals feeding, which implies that the rate of becoming more densely populated bee colonies is highest for 7-days intervals feeding during the research period. Similar findings were reported by (Kim et al., 2024), who observed that honeybees fed with nectar and pollen supplements due to insufficient supplies of nectars and pollens from the field during the dearth period, assist bee colonies to be more populated and productive in readiness for nectar flow period immediately after dearth period. (Kumar & Mall, 2018) identified that regardless of bee strength, all colonies fed sugar syrup had accelerated egg-laying activities. (de Oliveira et al., 2020) discovered that feeding sugar syrup increased egg laying and colony development.

#### Pollen cells

The total number of pollen cells were increased for all tested treatments. Although all treatments have increased the total pollen cells, the extent of the increment of pollen cells is different for each treatment as per supplementary Figure 3. Among all tested treatments, a 10-days intervals of sugar syrup feeding have increased the pollen cells by the greatest extent, i.e., by 21.34 times, followed by 15-days of feeding intervals, i.e., by 13.25 times. Among all treatments, the control treatment showed the lowest changes in the total pollen cells at the end of the experiment, i.e., by only 1.94 times, followed by 7-days of feeding intervals, i.e., only by 3.40 times. The less increase of pollen cells in case of 7-days feeding intervals may be due to a change in the grazing behavior pattern of the colony. Frequent application of sugar syrup to the beehives in 7-days of feeding intervals treatment may change honeybees to sluggish, where rather than grazing to natural nectars and pollens, the colony becomes more dependent on the applied sugar syrup. Several pollen cells differentially changed as per treatments. The total number of pollen cells varied in different fashions during each experiment week for different treatments. The lowest slope of





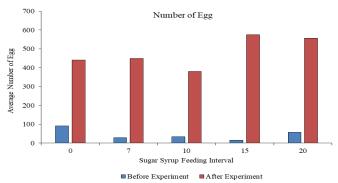


Figure 4. Effect of different feeding intervals on egg laying by queen bee.

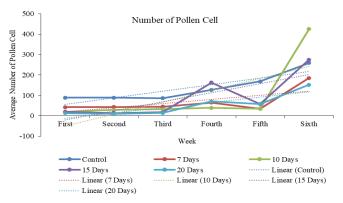


Figure 5. Trend line graph of number of pollen cells for all tested treatments.

the linear regression is observed for a 7-days feeding intervals, i.e., 20.264 (R<sup>2</sup>=0.4329), followed by a 20-days feeding intervals, i.e., 25.671 (R<sup>2</sup>=0.7561). 10-days feeding intervals of sugar syrup have the greatest slope of the linear regression line, i.e., 58.536 (R<sup>2</sup>=0.4622), followed by 15-days feeding intervals, i.e., 44.343 (R<sup>2</sup>=0.608). The results revealed that the pollen collection rate was more steady and rapid for 10-days feeding intervals, followed by 15-days intervals feeding. Similar to our result, pollen collection is higher for sugar syrup-fed colonies than for the control colonies. (Gemeda et al., 2018) and (Liu et al., 2020) have documented that sugar syrup-fed colonies show higher pollen cells. After feeding syrup, (Gemeda et al., 2018) discovered a 2.16-fold increase in sweet cherry pollen collection, a 3.27-fold increase in field bean pollen collection, and a 5.2-fold increase in red clover pollen collection. Sugar syrup has been shown to increase the amount of kiwifruit pollen collected by colonies (Estravis-Barcala et al., 2024). (Avni et al., 2009) also reported that pollen stores were higher in sugar syrup-fed colonies. For all measured major parameters with five tested treatments at the start and end of the experiment, average values are expressed with standard error in Tables 3 and 4.

Table 3. Status of measured	l parameters b	efore the	experiment.
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Treatments	Sealed brood area	Sealed honey area	Number of eggs	Number of pollen cells
0	28.25(±19.349)	385.875(±40.86124)	91.25(±70.97579)	88.25(±58.44709)
7	28.875(±28.875)	299.875(±74.62612)	28.75(±26.78736)	42(±37.74255)
10	23.5625(±23.5625)	492.3125(±52.40243)	34(±21.10687)	19(±10.22252)
15	13.6875(±11.39964)	402.625(±54.30139)	15.25(±15.25)	19.25(±10.38729)
20	28(±15.71226)	390.8125(±40.65882)	57.75(±47.10339)	12.75(±5.498106)

Table 4. Status of measured parameters after the experiment.

Treatments	Sealed brood area	Sealed honey area	Number of eggs	Number of pollen cells
0	116.625(±67.41766)	177.5625(±75.14641)	440.75(±238.9712)	259(±107.3856)
7	64(±39.91867)	168.6875(±65.03231)	449(±1136.8442)	184.75(±88.02119)
10	8.4375(±5.342806)	218.125(±71.99685)	380.5(±161.4505)	424.5(±281.1279)
15	86.0625(±46.38678)	204.625(±33.96161)	575(±117.5358)	274.25(±70.49867)
20	70(±70)	144.125(±63.01864)	556.25(±160.3955)	151.75(±62.15756)

# Conclusion

Artificial feeding during the winter dearth is vital for various bee activities, regardless of the feeding frequency. Among different feeding intervals used in farming practices, feeding at 15-days intervals proved to be the most effective. This practice helped preserve honey reserves in the hives and supported colony growth during the winter by increasing the number of eggs, sealed brood areas, and pollen cells, which are critical for maintaining healthy beehives. Although there may be an abundance of bee flora, low temperatures during winter significantly restrict foraging activity. Temperature plays a key role in influencing bee foraging behavior. Regarding honey reserves, hives fed at 7-days intervals stored the most honey, followed by those fed at 15-days intervals. On the other hand, the highest number of pollen cells, which reflect the foraging activity of colonies, was observed in hives fed at 10-days intervals. The greater number of pollen cells at 10-days feeding intervals compared to 7-days intervals could be attributed to less disturbance in hive temperature and more active colonies, as frequent feeding or an excess food supply in hives with 7-days intervals can cause inactivity. This study highlights that feeding sugar syrup as an artificial nectar supplement is essential throughout the winter dearth period. Farmers are advised to feed their bee colonies at 15days intervals, as this frequency effectively maintains optimal conditions for the colonies. During the two months of peak winter dearth in subtropical region of Nepal, farmers should feed their hives 4-5 times at 15-days intervals. This approach ensures better colony survival, maintenance, and overall performance.

# DECLARATIONS

# **Authors contribution**

Conceptualization: D.K., S.A., S.B. and K.K.; Methodology: D.K., S.B. and S.A.; Software and Validation: D.K., and S.A.; Formal analysis: D.K. and S.A; Investigation: D.K.; Resources: D.K.; Data curation: D.K. B.B and D.B.; Writing original draft preparation:

D.K.; Writing-review and editing: D.K., B.B. D.B., K.K.; Visualization: D.K.; Supervision: K.K., S.A., S.B.; All authors have read and agreed to the published version of the manuscript.

# **Conflict of interest**

There is no conflict of interest among all authors for the preparation and publication of this manuscript to this journal.

# **Ethics** approval

Ethics approval was not applicable to this research project, since the study did not involve any animal and human participant.

## **Consent for publication**

All co-authors have provided their consent to publish this manuscript in AAES.

# Data availability

Data will be provided upon request.

#### Supplementary data

Supplementary data are available.

#### **Funding statement**

This study did not receive any formal monetary support from governmental, private, or non-profit funding agencies.

#### Additional Information

No additional information is available for this paper.

# ACKNOWLEDGMENTS

Authors express their sincere gratitude to Mr. Dil Bahadur Gurung; apiary owner for his gigantic support, helping hands, and valuable assistance throughout field experiments.

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