Enhancing honey and bee bread cells number from Indonesian honeybee *Apis cerana* by feeding modification

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Abstract

The availability of both nectar and pollen sources as the honeybee feed is required to improve the health and development of colonies. Honeybee beekeeping is usually a scarcity of both nectar and pollen sources especially in the rain season that impacts on decreased productivity. The present study aimed to enhance honey and bee bread cell number from Indonesian honeybee *Apis cerana* by feeding modification. Thirty colonies of honeybee *A. cerana* from the traditional hive (*Glodok* hive) moved to box hive were divided into 6 groups treatments were A1B0 (sugar palm sap without sugar palm pollen); A2B0 (coconut sap without sugar palm pollen); A3B0 (50% sugar palm sap + 50% coconut sap without sugar palm pollen); A1B1 (sugar palm sap + sugar palm pollen); A2B1 (coconut sap + sugar palm pollen); and A3B1 (50% coconut sap + 50% sugar palm sap + sugar palm pollen). The results showed that 5 days after moving from *Glodok* hive to the box hive was the best time to open the queen cage was characterized by the high in foragers entrance hive, new combs length, and low absconding level of *A. cerana*. In addition, the feeding modification using sugar palm sap, coconut sap, sugar palm pollen, and their combination significantly affected the sap amount that can be collected by foragers, honey and bee bread cells number of *A. cerana* ($p<0.01$). The combination of coconut sap with sugar palm pollen was higher in sap can be taken by foragers, honey and bee bread cells than the other treatments.

Keywords: *beekeeping, box hive, coconut sap, pollen, sugar palm sap*

Introduction

Apis cerana is one of the Asian honeybee species that include medium size bees. The distribution of *A. cerana* in Asia is divided into six morphoclusters such as Northern *cerana*, Himalayan *cerana*, Indian plains *cerana*, Indo-Chinese *cerana*, Philippine *cerana*, and Indo-Malayan *cerana*. In Indo-Malayan *cerana* is distributed from southern Thailand, Malaysia, and Indonesia (Radloff et al 2011). Furthermore, *A. cerana* in Indonesia is distributed in several islands such as Kalimantan, Sumatera, Java, Bali, Sulawesi, Papua, Lombok, Sumbawa, and Seram (Hepburn and Radloff 2011; Radloff et al 2011). Beekeeping of *A. cerana* in Indonesia has been practiced by communities especially beekeepers around the forest and agricultural areas. Beekeeping can be used as an alternative livelihood because their products (honey, bee bread, royal jelly, and propolis) are high selling value and low cost to increase the beekeepers income. In addition, the main role of honeybee is the pollinator agent of plants and contributes significantly to increasing the plants productivity which impacts on improving livelihoods (Partap 2011; Pohorecka et al 2014). Furthermore, the beekeeping of *A. cerana* in Indonesia, especially in Java, Bali, Nusa Penida, and Sumbawa is important for income from activity generated from smallholder beekeepers (Schouten et al 2019).

In Lombok, the beekeeping of *A. cerana* is usually using a traditional hive from coconut stalks called *Glodok* hive (local name in Lombok). The empty *Glodok* hive is hung in trees located in the forest or garden to invite the *A. cerana* to build a new nest in the hive. The problem when beekeeping of *A. cerana* using *Glodok* hive is the difficulties when controlling colony development and harvesting their products. However, the main problem faced by beekeepers is the limited amount of bees feed on the plant flowers which can bloom all year. Schouten et al (2019) reported that several problems of beekeeping *A. cerana* in Indonesia such as the minimum of queen bees rearing, bee space understanding, bees absconding, the design of beehive has not standardized, honey has high moisture content and fermentation process. The feed sources of both nectar and pollen are the essential requirement when beekeeping is conducted. The nectar is obtained from plant flowers (floral nectar) and other parts of the plant like a leaf and leaf stalk (extrafloral nectar) (Abrol 2011; Pacini and Nicolson 2007; Agussalim et al 2018), while pollen obtained from the anther of plant flowers (Abrol 2011; Agussalim et al 2018). The nectar and pollen are used by the bee to produce honey and bee bread, respectively, and their availability is required to improve the development and health of the honeybee colony (Agussalim et al 2018).

One of the feed sources for honeybee *A. cerana* is sap (*neera*) which is obtained from the coconut and sugar palm. Coconut and sugar palm sap are available continuously around beekeeping locations, especially in North Duman village, West Lombok, Indonesia. These saps contain some nutrients that required by honeybee such as sugars, vitamins,

secondary metabolites, amino acids, volatile acids, minerals, and vitamins (Ho et al 2007, 2008; Borse et al 2007; Xia et al 2011; Pontoh and Smits 2015; Saputro et al 2019; Haryanti et al 2020; Hebbar et al 2020). Based on the chemical composition, these saps (coconut and sugar palm) have a potential for honeybee *A. cerana* feed, especially when the rain season is limited or lacking feed. The coconut and sugar palm saps are used as the feed honeybee *A. mellifera* has been studied by Erwan et al (2020) that the bees feed by coconut sap is higher in sugar reducing and lower in acidity than honey fed by sugar palm sap, while the moisture, sucrose content, and diastase activity are similar, however in honeybee *A. cerana* is not studied. Pokhrel et al (2006) reported that the beekeeping of *A. cerana* Fabricius from Chitwan Nepal with the forages mustard, *Brassica* spp, buckwheat, and *F. esculentum* is resulting honey and bee bread cells are highest is 6,739 cells/colony (ranged from 5,359 to 8,984 cells/colony) for honey cells and 1,104 cells/colony (ranged from 870 to 1448 cells/colony) for bee bread in the late December. Furthermore, in February is decreased 43.4% is 3,815 cells/colony (ranged 2,537 to 4,815 cells/colony) for honey cells and decreased 28.1% is 794 cells/colony (ranged 491 to 984 cells/colony) for bee bread cells because of the limited nectar availability and high in bee bread consumption to rearing brood cells. Therefore, the present study aimed to enhance the number of honey and bee bread cells from Indonesian honeybee *A. cerana* by feeding modification.

Materials and Methods

The colony selection technique

The fifteen colonies of honeybee *A. cerana* were used in this study was obtained from beekeepers in the North Duman village, Lingsar district, West Lombok, Indonesia. The colonies were selected based on the length and diameter of the coconut stalks from the traditional hive (*Glodok* hive). The *Glodok* hives have a similar size were 35 cm for length, the diameter was 14 cm, and the combs were 4 frames (Photo 1). The colony of *A. cerana* from *Glodok* hives were moved to box hives was done by taking 4 combs and then was tied to a comb frame (length was 35 cm and height was 22 cm). Afterwards, the queen bee was put into a cage made from wire netting (length was 8 cm and the diameter was 3 cm) and then the queen cage was tied to a comb frame was shown in Photo 2. The size of box hives were length 40 cm, width 30 cm, and height 26 cm. All colonies were moved from *Glodok* hive to box hives (workers, drones, and larvae). The treatment in this study was time of queen bee cage opened consists of 3, 5, and 7 days after the queen bee was put in the cage.

Photo 1. The colonies of honeybee *A. cerana* in *Glodok* hive

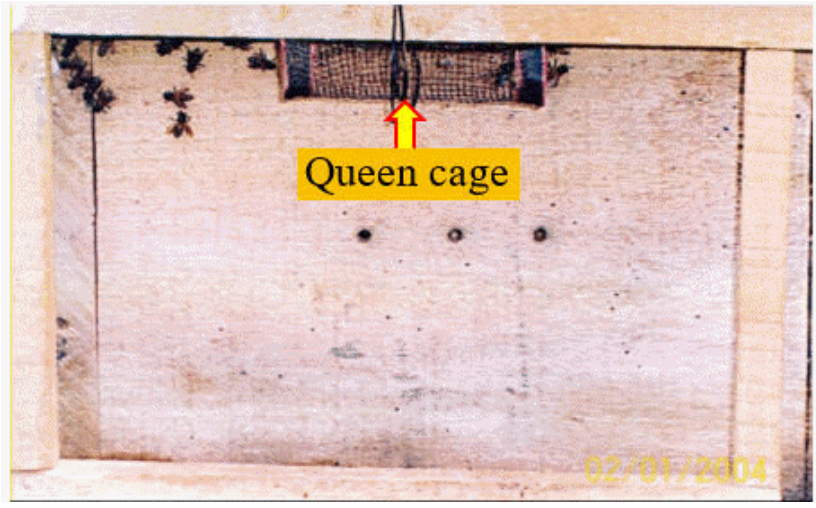

Photo 2. The queen cage for honeybee *A. cerana* was used in the study

Foragers number entrance hives, combs length and absconding level

The number of foragers entering hives was counted using a hand counter check for all foragers. To make it easy to count the foragers, the entrance hole was narrowed so the foragers can be seen clearly. The new combs were formed by workers in the box hives and can be seen in color and combs size. New combs usually were white in color and thin. The length of combs was measured using a ruler. The absconding level of the bees (queen bee, workers, and drones) from box hives totally was characterized by them were flying to find the new hive in the wild and the box hive was checked to verify the honeybee condition.

The given technique of plant sap

The fresh sugar palm (*Arenga pinnata*) and fresh coconut (*Cocos nucifera*) saps (*neera*) were used in this study and were collected in the morning from farmers in North Duman village, Lingsar district, West Lombok, Indonesia. Afterwards, the saps were divided into two containers consists of the plastic plate (diameter was 15 cm) was completed with 4 to 5 twigs as the perch of foragers when they collect sap and split bamboo (length was 20 cm and diameter was 7 cm) (Photo 3). In this study, 5 colonies of honeybee *A. cerana* were placed in front of the box hive with placed plastic plate and split bamboo that was filled with coconut and sugar palm saps. When the foragers collect coconut and sugar palm saps were counted using hand a counter the number of foragers who collect the sap in one day. In addition, the chemical composition of coconut and sugar palm saps was determined using proximate analysis were moisture, protein, crude fiber, and fat (AOAC, 2005) and sucrose content (AOAC, 2005).

Photo 3. Plastic plate and split bamboo were used to hold the coconut and sugar palm saps

To determine the behavior of *A. cerana* foragers when they fly to visit the plastic plate when collecting sap, 9 colonies were used. The coconut or sugar palm saps volume was given in the morning for each colony was 250 ml gradually because based on the previous study that the honeybee *A. cerana* was collect maximum 200 ml of sap in one day and the plate plastic was placed in front of an entrance at the distance were 0.5, 1, and 1,5 m, respectively. The sap amount collected by foragers was counted using the equation: the number of saps given reduced by the rest of the saps in a plastic plate or split bamboo and then was measured using beaker glass. The distance of plastic plate and split bamboo was 600 meters from each hive in order the foragers not to collect sap and pollen from other treatments except in front of their hive.

The given technique of sugar palm pollen

Sugar palm pollen used in this study was obtained around the North Duman village, Lingsar district, which was cut from sugar palm flower stalks. Pollen from sugar palm was placed beside plate plastic contain sap with hung the sugar palm pollen at wood with the height 1.5 m and placed in front of *A. cerana* hive with a distance 1 m. In addition, sugar palm pollen also was placed above the hive (Photo 4).

Photo 4. Sugar palm pollen hung at wood (left) and above the box hive (right)

The number of foragers when collecting pollen from sugar palm stalks was counted using a hand counter check for 5 minutes. In addition, the chemical composition of pollen was determined using proximate analysis were protein, fat, crude fiber, and ash (AOAC 2005).

Bee bread and honey cells number

Bee bread and honey cell numbers (Photo 5) were counted using a hand counter check every 3 weeks for 3 months of beekeeping.

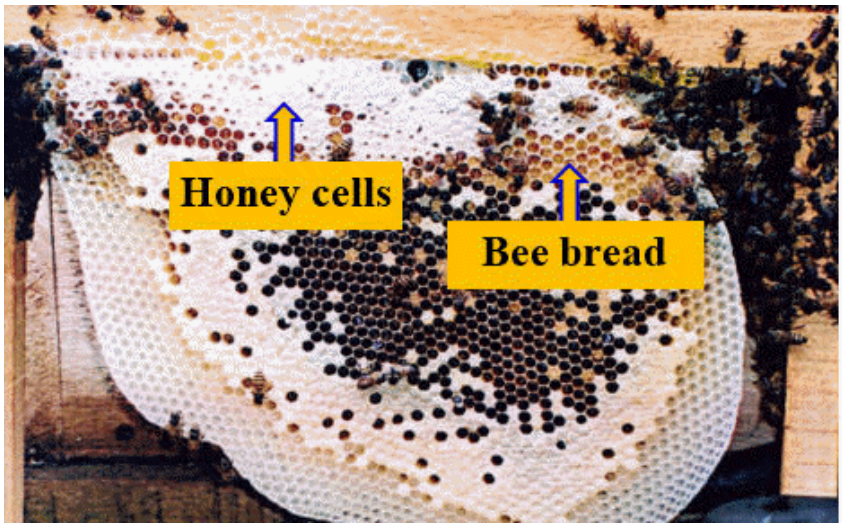

Photo 5. Honey and bee bread cells from *A. cerana*

Study design and analysis statistical

In this study was used randomized complete block design (RCBD) was used with factorial 3x2 and 5 replicates (30 colonies). The first factor was plant saps (A1 was sugar palm sap, A2 was coconut sap, and A3 was 50% of sugar palm sap + 50% coconut sap). The second factor was without sugar palm pollen (B0) and with sugar palm pollen (B1). The data were analyzed by analysis of variance based on RCBD and contrast orthogonal tests (Steel et al 1997).

Results and discussion

Colony condition

The present study showed that the number of brood cells of *A. cerana* was ranged from 225 to 250 cells per colony. The time 5 days after the colony moved from the *Glodok* hive to the box hive is the best time to open the queen cage than 3 and 7 days. The time 5 days showed that the foragers very active to collect the food from the nature that characterized by

the high of foragers number was 2,585 heads entrance to hive. Furthermore, the new combs were formed with white color and thin with the length was 8 cm. In addition, it indicates that the colony has started adaptation to stay in the box hive with the absconding level was 20% and lower than in 3 and 7 days (Table 1). The different foragers number can be entrance hive in each time to open the queen cage might be caused by the different workers population, however in our study not counted them.

In addition, this condition might be occur the communication among the colony members (workers and drones) with the queen by pheromone hormone that is secreted by mandibular gland of the queen bee. Through this compound, all the activities of honeybees are controlled such as the feeding in the colony (queen, drones, and larvae) and as the danger alarm when they are attacked by an enemy or pest (Sihombing 2005). The main component of pheromone from queen bee such as 9-oxodeconic and 10-hydroxydeconic acids that roles to controlled the biology and activity colonies, inhibit the development of workers ovarium and inhibit the biosynthesis of juvenile hormone (Hepburn 1992; Kaatz et al 1992).

Table 1. The foragers number the entrance hive, new combs length, and absconding level from different times to open the queen cage

Parameters	Time to open the queen cage		
	3 days	5 days	7 days
Foragers number entrance hive (heads)	961	2,585	1,473
New combs length (cm)	-	8	8
Absconding level (%)	80	20	80

The times 3 and 7 days to open the queen cage in box hive is higher in absconding level was 80%. The high in absconding might be caused by the food lacking in the hive because its activity of foragers to entrance hive was 961 and 1,473 heads were lower, respectively (Table 1). Hepburn (2011) explained several factors are causing the absconding in honeybees such as climate (high temperature, extreme aridity, and extended cold or rains), seasonal related to the availability of plant flowers for nectar and pollen sources, and the colony growth. Pokhrel et al (2006) explained that the major cause of *A. cerana* absconding is feed scarcity (nectar and pollen) and the scarcity of bee bread in the hive. The bee bread scarcity is causing the low rearing of brood cells and accelerated the absconding of *A. cerana*. Furthermore, that the sugar candy feeding for three weeks can be stopped absconding and improved honey storage 171%. Generally, in Java, Indonesia the beekeepers of *A. mellifera* are using the sugarcane that is diluted into water to feeding them when the food lacking, especially in the rain season to decrease the absconding level of *A. mellifera*.

The given technique of plant sap

The results showed that plate plastic containing coconut or sugar palm saps is the higher of foragers to collect sap is ranged from 10 to 20 foragers than in split bamboo is ranged from 1 to 2 foragers. In the plastic plate it is easy for foragers to collect sap because it is completed by 4 to 5 twigs as the perch of them when collecting the sap and to avoid the wet wings and drowning that impacted on their death. In addition, the distance of 1 meter to place the sap feeder from the box hives was an effective distance that characterized the quick time of foragers to collect sap. Based on the behavior observed in the distance 1 meter showed that foragers were directly visit and perch to plastic plate than in distance 0.5 and 1.5 meters.

The chemical composition of sugar palm sap showed that it contained a moisture content was 90.12%, protein 0.14%, crude fiber 0.02%, fat 0.21%, and sucrose 9.23%. Furthermore, the coconut sap contained a moisture content was 86.26%, protein 0.30%, crude fiber 0.01, fat 0.17, and sucrose 13.15%. Mogea et al (1991) reported that sugar palm sap contains 12 to 15% of sugar. These results showed that coconut sap was higher in sucrose content than sugar palm sap. In addition, the sucrose content from sugar palm sap in our study is differ than reported by Pontoh and Smits (2015) is ranged from 10 to 15%. The fresh coconut sap contains many nutrients such as amino acids, acids (acetic, lactic, tartaric, citric acid, and malic), sugars, vitamin C, and phenolic compounds (gallic acid, caffeic acid, protocatechuic acid, p-coumaric acid, and galangin) (Xia et al 2011), and volatile acids (Borse et al 2007; Xia et al 2011). Therefore, based on the chemical composition the coconut sap can be used as the main feed the bee *A. cerana* or other bees, but it needed advanced study especially production and quality of honey.

The given technique of sugar palm pollen

The results showed that the sugar palm pollen was hung in the log with height 1.5 meters from the ground and was much more visited by the *A. cerana* foragers to collect pollen than was placed above the box hive. The number of foragers that collect pollen from hung sugar palm was higher and ranged from 30 to 40 heads/5 minutes than was placed above the hive was ranged 5 to 10 heads/5 minutes. The hung sugar palm pollen will be easy for foragers to taken pollen grains because the flowers can be perfectly broken, while the sugar palm pollen was placed above the box hive was more fallen in the ground because of overlap each other and does not perfectly break impact on the difficulty of foragers to collect pollen (Photo 6).

The chemical composition of sugar palm pollen contains protein 27.12%, fat 4.27%, crude fiber 0.94%, and ash 4.67%. Based on protein content, it was shown that sugar palm pollen is one of the quality pollen sources for honeybees,

especially for *A. cerana* in our study. Sadapotto et al (2019) reported that sugar palm (*Arenga pinnata*) was found the dominant pollen source from bee bread produced by honeybees *A. dorsata, A. mellifera*, *A. cerana*, and the stingless bee (*Tetragonula* sp.). It indicates that sugar palm pollen is the potential pollen source for honeybee especially in the rain season when feed is lacking.

Photo 6. Sugar palm pollen imperfectly breaks (left) above the box hive and perfectly breaks that hung in log (right) (red circles showed the foragers of *A. cerana* when collect pollen).

Sap amount collected by foragers

The results showed that the feeding modification using sugar palm sap, coconut sap, sugar palm pollen, and their combination were affecting the sap amount that can be collected by *A. cerana* foragers (Table 2). The sap can be collected by foragers ranged from 5,327 to 8,715 ml/colony of *A. cerana* (Table 2). The combination of coconut sap and sugar palm sap with sugar palm pollen was increased the sap amount can be collected by foragers than other treatment, however the best combination was coconut sap with sugar palm pollen. The sap amount is related to the aroma, flavor, and sugar content. The coconut sap has a sweet flavor, fresh aroma, fragrant, and the changes from fresh into sour (natural fermentation) was slower than sugar palm sap and their combination, therefore the coconut sap was much more can be collected by foragers.

The natural fermentation in sap is related to higher in moisture content, rich sugars content is ranged 10 to 15%, and nearly neutral in pH which is a good media for microorganisms growth (Xia et al 2011). In addition, in the coconut sap is found 166 yeasts isolates, 39 bacteria isolates, and the acids (acetic, lactic, citric, and malic acids) and the main volatile acid is acetic acid (Xia et al 2011) and 74 lactic acid bacteria isolates (Somashekaraiah et al 2019).

Table 2. The sap amount, honey and bee bread cells number of honeybee *A. cerana*

Parameters	Treatments						SEM	*p*
	A1B0	A2B0	A3B0	A1B1	A2B1	A3B1		
Sap amount (ml/colony)	5,327^{d}	6,518^{b}	5,659^{c}	6,297^{b}	8,715^{a}	6,372^{b}	226.6	0.000
Honey cells number (cell/colony)	874^{e}	1,760^{c}	1,364^{d}	2,198^{b}	2,713^{a}	1,722^{c}	122.5	0.000
Bee bread cells number (cell/colony)	731^{f}	830^{e}	858^{d}	1,562^{a}	1,414^{b}	1,068^{c}	68.5	0.001

^{a,b,c,d,e,f} *Different superscripts within rows indicate differences at p<0.05 Abbreviations: A1B0 (sugar palm sap without sugar palm pollen); A2B0 (coconut sap without sugar palm pollen); A3B0 (50% sugar palm sap + 50% coconut sap without sugar palm pollen); A1B1 (sugar palm sap + sugar palm pollen); A2B1 (coconut sap + sugar palm pollen); A3B1 (50% coconut sap + 50% sugar palm sap + sugar palm pollen)*

The physical and chemical changes from sap can occur when they are tapped until they are given to honeybee *A. cerana* as the feed. The sap will be changed in a chemical process in three steps, the first is changes of sucrose into glucose and fructose by invertase enzyme that produced by the microorganism. The second, glucose and sucrose components will undergo the fermentation process by the microorganism into alcohol ethyl. The last step is alcohol ethyl oxidized into acetate acid by acetic acid bacteria (Chinnamma et al 2019). The sour sap is characterized by the whiter and turbid colors, aroma, and flavor is the sour impact the foragers dislike, resulting in the sap amount can be collected by the foragers is low. The honeybee is very selective when visiting and take the feed from plant flowers especially when collecting nectar (floral nectar) and extrafloral nectar is affected by aroma, sugar content, and several inhibit factors such as pest, insecticide or pesticide, and toxic compound in the nectar content (Abrol 2011; Pacini and Nicolson 2007). Furthermore, based on shapes, colors, and odors of flowers, the requirement of energy, caloric rewards, temperature, humidity, solar

radiation, light, and nectar production (Abrol 2011). In addition, the sugar content of coconut sap was higher (13.15%) than sugar palm sap (9.23%).

The honey and bee bread cells number

The results showed that the honey cells number from each colony of *A. cerana* was different and was ranged from 874 to 2,713 cells/colony (Table 2). The combination of coconut sap with sugar palm pollen was resulted the higher in the number of honey cells produced by workers in the hive than other treatments. The different honey cells number was produced in each colony of *A. cerana* is related to sap amount that can be collected by foragers, where the combination of coconut sap with sugar palm pollen was higher than the other treatments (Table 2). The nectar in this study was replaced by sap and pollen from sugar palm flowers as the source of sugars (mainly fructose, glucose, and sucrose), protein (amino acids and enzymes), fats, minerals, and vitamins which required by honeybee growth and developed, to repairing the worn out of tissue, and to stimulate hypopharyngeal gland development (Abrol 2011). The sap that collected by foragers will be transferred to other workers in the hive to produce honey. The honey production process starts when foragers have been collecting nectar and storage in the honey sac of their abdomen. Afterwards, the foragers move the nectar from their abdomen to another worker (in one or several workers) in the hive with opened their mandibles with pulling on their proboscis, and transferred the nectar from their honey sac to the worker receiver. The worker receiver will extend the proboscis and sucking the nectar, and this transfer quickly depends on bee age, honeybee species, colony strength, and the availability of raw materials, and then used to produce honey in their hive (Hart and Ratnieks 2001).

The honey cells number was higher in the combination of coconut sap with sugar palm pollen in our study might be caused the food amount entranced to the hive is exceeded their requirement for maintenance (workers, drones, and larvae) and colony growth, therefore the exceed food is stored as honey in the combs. Therefore, it stimulates the workers who work in the hive to increase the production of empty cell using wax that is used to honey deposit. In addition, the added sugar palm pollen is affected significantly by the formation of honey cells and is related to pollen availability as the main source of protein. In our study, the protein content of sugar palm pollen is high 27.12% might contribute to fulfill the protein requirement of *A. cerana,* especially amino acids. The honey cells number produced by workers is affected by the foragers number, worker population, activity rate of foragers, the availability of nectar and pollen sources, temperature, humidity, light, and solar radiation (Abrol 2011). The honey cells number in our study (Table 2) differs with reported by Pokhrel et al (2006) that honeybee *A. cerana* Fabr. from Chitwan Nepal during December to January with the forages mustard, *Brassica* spp, buckwheat, and *F. esculentum* where the honey cells number is highest in the late December is 6,739 cells/colony (ranged from 5,359 to 8,984 cells/colony), while in February is decreased 43.4% is 3,815 cells/colony (ranged from 2,537 to 4,815 cells/colony).

The present results showed that the combination of sugar palm (sap + pollen) and coconut sap with sugar palm pollen was higher in bee bread cells number than in other treatments and the bee bread cells number was ranged from 731 to 1,562 cells/colony (Table 2). The high of bee bread cells is related to the distance among the sugar palm pollen placed from the hive, the activity rate of foragers to collect nectar and pollen, workers population, however workers population is not studied. The distance of 1 meter is easing the foragers to collect and take pollen from sugar palm so increasing the trip frequency per unit time to take pollen is high. In the hive, pollen is mixed with honey, bee secretion, and then undergoes fermented by lactic acid to be preserved and stored in the combs (Bogdanov 2017). Generally, the worker will place the bee bread nearby with brood cells to ease the adult newborn to take bee bread as the feed. The bee bread cells number in our study (Table 2) differs from the reported by Pokhrel et al (2006) that honeybee *A. cerana* Fabr. from Chitwan Nepal during December to January when the forages *Brassica* spp., mustard, *F. Esculentum*, and buckwheat are full blooming. The highest bee bread storage is 1,104 cells/colony (ranged from 870 to 1,448 cells/colony) in late December, while in February is decreased 28.1% is 794 cells/colony (ranged from 491 to 984 cells/colony) because of the high in bee bread consumption to rearing brood cells. The availability of bee bread in the nest is negatively correlated with *A. cerana* absconding and less bee bread in the nest is increasing the absconding honeybee *A. cerana*.

Conclusions

- The combination of coconut sap with sugar palm pollen enhances the honey and bee bread cells number and sap can be collected by foragers from honeybee *A. cerana*.
- The 5 days after honeybee *A. cerana* moved from *Glodok* hives to the box hives is the best time to open the queen cage it increased the foragers entrance to the hive, the new combs length, and the low absconding level.
- The coconut sap and sugar palm pollen can be used as the sustainable feed for honeybees when they are scarcity of the feed especially in the rain season which will still observe the availability of nectar from plant flowers around beekeeping locations.

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