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The influence of queen bee age, the number of brood combs, and the use of a queen excluder on comb brood size in *Apis mellifera* bees during the blossom season

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Abstract

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This study was carried out in the village of Kayoman, Wonorejo District, Pasuruan Regency, Indonesia. The aim of the study was to develop colony management and to prepare for the main honey harvesting in order to obtain optimum broods. The results indicated that the cumulative size of the S1 brood combs was different from those of S2. There were no significant differences between presence (P1) of five brood combs (S1) and (S2), but P1 (S1) is different from P2 (S1) and absence (P2) of three brood combs (S2). The relative size of the S2 was different from the S1. The results also indicated that the cumulative brood quality was depended on the number of brood combs, and the quality of S1 was different from S2. Brood comb size was depended on the number of brood combs and the use of a queen excluder. Brood comb quality depended on the age of the queen and the number of brood combs. In order to increase colony honey production during the blossoming season, keepers should maintain bees at a maximum age of three months (R3), the number of brood combs should not exceed three, and a queen excluder should be used. A bee colony management system should be based on the above findings.

Keywords: brood; honey pollen; number of combs; queen bee age; queen excluder
Abbreviations: SE – standard error; p – probability; P – queen excluder in brood comb (k = 1, 2); P1 – present; P2 – absent; qe – queen excluder; R – age of queen bee (i = 1, 2, 3); R1 – bees age 11-12 months; R2 – bees age 6-7 months; R3 – bees age 2-3 months; S – brood comb (j = 1, 2, 3); S1 – five brood combs; S2 – three brood combs; SE – brood combs; SK – unoccupied combs; Y – obtained data; ε – experimental error (m = 1, 2, 3 for all i, j, and k); μ – general average

Introduction

Nectar and pollen are plant products that are the main feed for honeybees (Sonter et al., 2019). Various types of plants produce nectar and pollen at different times; however, because Indonesia is a tropical country, feed for honeybees are available throughout the year (Schouten et al., 2019). In the beginning of the dry season, many flowering plants produce nectar and pollen that give abundant feed to bees. The cotton plant (*Ceiba pentandra*) is an example of a potential type of bee feed that produces nectar and pollen. To provide bees with an environment in which they can make optimal use of available nectar and pollen during the blossom season, consideration must be given to the age of the queen, the number of brood combs, the use of a queen excluder, and overall rearing management (Ambrose, 1992).

The queen bee is the only bee caste inside of the colony that produces eggs (Renzi et al., 2016). A queen bee that is young has low reproductive activity, but that activity will increase with age. To date, bee farmers have not identified the optimal age of the queen bee in order to prepare the colonies for the blossom season. The addition of the number of brood comb is done by combining small colonies and removing the queen bee (Walton et al., 2018). As a consequence, the number of colonies cultivated by bee farmers has decreased. For those reasons, bee farmers need to consider the exact number of brood comb needed in the colony to produce a strong colony (Horn, 1998).

During the blossom season, big and small colonies will continue to extend the size of brood comb which is gathered in the comb (Nurnberger et al., 2018). Honey harvesting on the brood comb during the blossom season creates space for the queen bee to lay eggs, but the production of harvested honey will be mixed with the release of grubs from the brood comb (Crane, 1980). This problem can be overcome by separating the queen bee from the honeycomb using a queen excluder. The queen bee will lay eggs in all of the comb hives inside of the hive box. It will affect the density of the broods (Barganska et al., 2015). A queen excluder is positioned vertically with a certain number of brood combs, and the excluder will keep the queen bee in a vacant position to lay new eggs. Quantitative analysis is needed to determine of these measures will cause the productivity of the broods to improve (Hayes, 1985).

The first aim of this research is to increase the productivity of the honey bee colony, *A. mellifera*, during blossom season by determining the age of the queen bee, the number of brood combs, and the impact of using a queen excluder in the beginning of blossom season. The second aim is to determine the selection of the honey bee, *A. mellifera*, farming method from the research result which was kept by bee farmers.

This research was conducted for two main reasons. The first was to determine the relationship between the age of the queen bee, the number of brood combs, and the impact of using a queen excluder. Understanding those factors would allow us to characterize the development of the broods honeycomb size, honey and pollen; the quality of the broods within a colony; and any changes in the weight of honey bee colony. The second reason was to understand differences in the production of broods, honey, and pollen between different bee farmers based on the research results.

Methods

Preparation of sample

This research was conducted in Kayoman village, Wonorejo sub-district, Pasuruan regency. An artificial queen bee from an *A. mellifera* colony was used. There were 36 colonies of the honey bee *A. mellifera*. There were 180 unoccupied honey combs and 144 cells of brood combs. The queen excluder was made of lined wire with a plastic frame, and it was 0.5 cm in length.

Experimental design

The following conditions were used: queen bees at the ages (R) of 11–12 months (R1), 6–7 months (R2), and 2–3months (R3); five (S1) and three (S2) brood combs (SE); and the presence (P1) or absence (P2) of a queen excluder (qe). Number of brood combs (SE), unoccupied combs (SK), and the presence of the queen excluder (qe) in the colony can be explained as follows:

- 5 SE+ qe + 4 SK(P1(S1));
- 5 SE+ 4 SK(P2(S1));
- 3 SE+ qe + 6 SK (P1(S2));and
- 3 SE + 4 SK (P2(S2)).

The arrangement order of the brood comb removal can be explained as follows:

- **SE1 +SE2 + SE3+qe +SK1** + SK2 + SK3 + SK4 + SK5 + SK6;
- SE2 +SE3 +***SK1***+ qe+***SE1*** + **SK2** + SK3 + SK4 + SK5 + SK6;
- SE3 +SK1 + ***SK2***+ qe+***SE2*** + SE1 +**SK3** + SK4 + SK5 + SK6;
- SK1 +SK2 +***SK3*** + qe+***SE3*** + SE2 + SE1 + **SK4** + SK5 + SK6;
- SK2 +SK3 +***SK4*** + qe+***SK1*** + SE3 + SE2 + SE1 + **SK5** + SK6;
- and so on following the addition of extra comb as mentioned previously.

Data collection

The cumulative size of the honey comb was determined by adding the initial size of honeycomb and the increase in the honeycomb size for each observation. The relative size of honeycomb was determined by multiplying the initial size of the honeycomb by 100% and then subtracting that product from the cumulative size of the honeycomb. With regard to assessing the brooding quality, cumulative brood honeycomb quality was determined by adding the new quantity from each day of observation to the initial quantity of the brood honeycomb. The relative brood honeycomb quality was determined by multiplying the quantity of the initial brood honeycomb by 100% and then subtracting that product from the cumulative quantity.

We measured several variables related to broods honeycomb size and brood quality. To capture the development of the brood honeycomb size, the size of the brood honeycomb per comb and per colony were measured at the beginning of the blossom season, and the cumulative brood honeycomb and the cumulative and relative size of honeycomb were measured during blossom season. To capture the brooding quality, the cumulative and relative brood quality were measured at the beginning of the blossom season and during the blossom season observational period.

Data analysis

The age of the queen bee, the number of brood combs, and the presence of a queen excluder was analyzed using a nested factorial design (Steel & Torrie, 1980; Sudjana, 1980). The following linear model was used:

$$Y_{(ijk)} = \mu + R_i + S_j + RS_{ij} + P(S)_{k(j)} + RP(S)_{(ik(j)} + \varepsilon_{m(ijk)}$$

The observation results were analyzed using covariance to determine the error normality, error homogeneity, accuracy of companion variable, and whether homogeneity of regression fulfilled the requirement (Steel & Torrie, 1980; Sudjana, 1980). If there was a difference in covariance analysis, it was tested using a least significant difference test (5%).

The observation result during the blossom season, regardless of the colony condition at the beginning of blossom season, was analyzed using variance analysis. Before the data were analyzed, error normality and error homogeneity tests were performed (Steel & Torry, 1980; Sudjana, 1980). If the result of data analysis indicated any differences, a follow-up test was done using a least significant difference test (5 %).

Results

Development of broods honeycomb size

At the beginning of the blossom season, the size of brood honeycomb per comb was the same for all treatments ($p > 0.05$). The size of the brood honeycomb per colony was significantly different ($p < 0.01$). Size of the S1 was wider compared to S2. During the blossom season, the cumulative brood honeycomb size depended on the brood honeycomb size in the beginning of blossom season ($p < 0.05$). There were no significant differences between the age of the queen, the number of brood combs, or the presence of the queen excluder ($p > 0.05$). There were also no significant results for the interaction with the cumulative brood honeycomb size ($p > 0.05$) (Table 1).

Table 1. The size of brood honeycomb per colony in the beginning of cotton plant season

The size of brood honeycomb per colony	
Treatment	Average (cm^2)
S1	2351.17 ± 118.441 a
S2	1380.65 ± 73.368 b

Note: number which be followed by same letter in every column is not different significantly ($p > 0.05$)

Role of brood comb and queen excluder

Regardless of the brood honeycomb size per colony at the beginning of the blossom season, the cumulative size of the brood honeycomb was significantly different ($p < 0.05$). These results were dependent on the queen excluder and the number of brood comb. While the relative size of the brood honeycomb was significant ($p < 0.01$), this finding depended on the number of brood combs. Size of the brood honeycomb in the bee colony with five brood combs and a queen excluder (P1 (S1) and P1 (S2)) was the same as the colony with three brood comes and an excluder (P1 (S1) and P2 (S2) (Table 2).

Table 2. The role of brood comb and queen excluder in the cumulative brood honeycomb size during cotton plant season

Cumulative Size of Brood Honeycomb			
Treatment	Average ± (SE) (cm^2)	Treatment	Average (cm^2)
P1(S1)	3708.48 ± 286.709 a	S1	3332.37 ± 202.997 a
P1(S2)	3115.29 ± 197.400 ab	S2	2857.37 ± 153.535 b
P2(S1)	2956.26 ± 239.931 b		
P2(S2)	2599.45 ± 211.157 b		

Note: number which be followed by same letter in every column is not different significantly ($p > 0.05$)

Role of the number of brood comb and brood quality per colony

Besides the queen excluder in the brood comb, the size of the brood honeycomb depended on the number of combs in the colony. S1 were wider than S2, resulting in S1 was still wider than S2 during the blossom season (Table 3). The quality of the brood honeybee comb per comb was not significantly different ($p > 0.05$). The quality of the brood honeybee comb per colony

Table 3. The role of the number of brood comb in the size of brood honeycomb size during cotton plant season

Relative Size of Brood Honeycomb	
Treatment	Average (%)
S2	113.50 ± 12.881 a
S1	42.28 ± 6.645 b

Note: number which be followed by same letter in every column is not different significantly ($p > 0.05$)

Table 4. Brood quality per colony in the beginning of blossom season

Brood quality per colony			
Treatment	Average ± SE	Treatment	Average
R3	31.75 ± 2.603 a	S1	37.61 ± 0.672 a
R1	29.83 ± 2.131 b	S2	23.00 ± 0.511 b
R2	29.33 ± 29.333 b		

Note: number which be followed by same letter in every column is not different significantly ($p > 0.05$)

depended on the age of the queen bee ($p < 0.05$) and the number of brood combs ($p < 0.01$) (Table 4).

The cumulative brood quality during the blossom season did not depend on the brood quality per colony at the beginning of blossom season. The brood quality during the blossom season was significantly different ($p < 0.01$) depending on the interaction between the age of the queen bee and the number of brood combs. The result show R3S1 was not different from the R1S1 or R2S1. R1S1 and R2S1 were not different from R2S1, R1S2, R1S2. However, R3S1 was significantly different from the R2S2, R3S2, and R1S2 (Figure 1; Table 5).

The quality of the cumulative brood during the blossom season, regardless of the quality of the brood per colony at the beginning of blossom season, was significantly different ($p < 0.05$) depending on the interaction between the age of the queen bee and the number of brood combs. The quality of the relative brood was significant ($p < 0.01$) depending on the number of brood combs. The quality of the cumulative brood depends on the interaction between the age of the queen bee and number of brood combs. R3S1 and R3S2 produced the same quality of brood. There were no significant differences between R1S1 and R2S1 compared to R3S2 and R3S2 (Figure 2; Table 6). The relative brood quality is for all of the experimental scenarios which depend on the number of brood combs. The relative brood quality in S2 was higher than S1. Nevertheless, in the beginning of the blossom season, S1 was of a higher quality compared to S2 (Table 7).

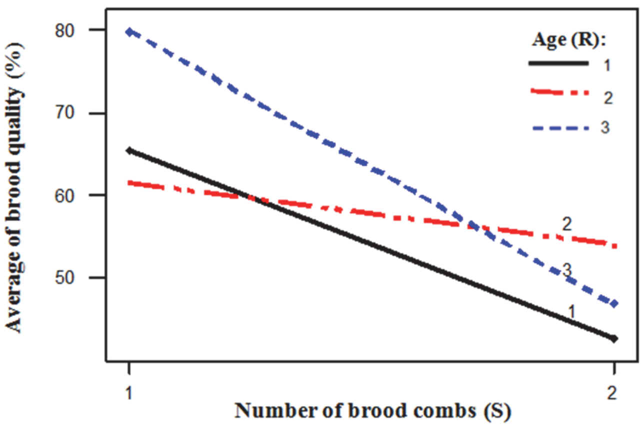

Fig. 1. Interaction between queen bee's age and number of brood combs towards the cumulative brood quality

Note: R: *1*: 1 year, *2*: 6 months, *3*: 3 months; *S*: *1*: 5 combs, *2*: 3 combs

Table 5. Interaction between queen bee's age and number of brood combs towards the cumulative brood quality during blossom season

Cumulative Brood Quality	
Treatment	Average ± (SE)*)
R3S1	80.02 ± 4.307 a
R1S1	65.46 ± 2.729 ab
R2S1	61.46 ± 2.816 ab
R2S2	53.90 ± 4.255 b
R3S2	46.58 ± 2.471 b
R1S2	42.41 ± 4.647 b

Note: number which be followed by same letter in every column is not different significantly ($p > 0.05$)

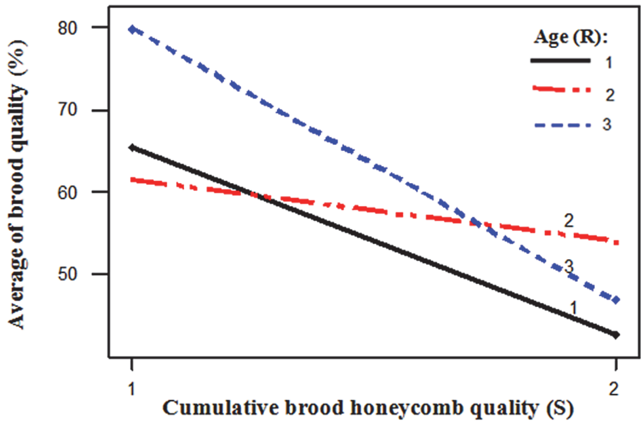

Fig. 2. Interaction between queen bee's age and number of brood comb towards cumulative brood honeycomb quality during blossom season

Note: R: *1*: 1 year, *2*: 6 months, *3*: 3 months; *S*: *1*: 5 combs, *2*: 3 combs

Table 6. The role of number of brood comb and interaction between queen bee's age and number of brood comb in cumulative brood honeycomb quality

Cumulative Brood Quality			
Treatment	Average ± SE	Treatment	Average
R3S1	70.00 ± 4.509 a	S1	68.98 ±2.659 a
R2S2	61.83 ± 4.881 ab	S2	47.63 ±2.412 b
R1S1	59.17 ± 2.039 b		
R2S1	55.50 ± 2.680 b		
R3S2	53.67 ± 2.859 b		
R1S2	49.67 ± 4.046 b		

Note: number which be followed by same letter in every column is not different significantly ($p > 0.05$)

Table 7. The role of number of brood comb in the quality of relative brood honeycomb

Quality of Relative Brood Honeycomb	
Treatment	Average (%)
S2	142.65 ± 14.133 a
S1	63.78 ± 5.655 b

Note: number which be followed by same letter in every column is not different significantly ($p > 0.05$)

Based on the results of this study, the good quality of the cumulative brood involved the three-month-old queen bees and the five brood combs. In contrast, the relative brood quality involved three-month-old queen bees and three brood combs. Therefore, the best quality of brood during blossom season was obtained from three-month-old queen bees and hives with three combs.

Discussion

Size of the brood honeycomb in the bee colony with five brood combs and a queen excluder was the same as the colony with three brood comes and an excluder (Matsuda & Sugiura, 2018). It was similar (five brood combs and a queen excluder) because during the blossom season the queen bees were at the same place which was equipped with a queen excluder and unoccupied combs. Thus, the chance for the queen bees to lay eggs and to produce the same brood honeycomb size was similar. According to Barth (1956) and Schmidt & Buchmann (1992), during the blossom season, the nectar and pollen are abundant, which rapidly increases the broods and which causes the empty brood combs to quickly become filled with eggs by the queen bee. However, the colony with three brood combs and a queen excluder was the same as the colony with three brood combs and no queen excluder, so the development of the brood honeycomb size during the blossom season increased.

The relative sizes of the brood honeycombs, as presented in Table 3, showed that the number of honeycomb with three and five combs were different. Referring to Table 1, the size of the brood honeycomb with the three combs at the beginning of the blossom season was smaller compared to the five combs. When there was an abundance of fresh pollen during the blossom season for the worker bees to bring into the colony as feed ingredients, the activity of the colony members increased. According to Morse & Hooper (1985) and Adeonipekun et al. (2016), fresh proteins in the pollen can improve the reproduction process of the queen bees, resulting in the increase in the relative brood honeycomb size with three combs. We found that it was better to use a colony with three brood combs and a queen excluder to increase the size of brood combs.

The experiment consider the size of the brood honeycomb in the beginning of the blossom season, the number of brood combs and the use of a queen excluder towards the production of cumulative and relative size of honeycomb during blossom season (Hernando et al., 2018). The three-month-old queen bee was not different from the one-year-old queen bee or the six-month-old queen bee with five brood combs. The one-year-old and the six-month-old queen bees with five combs were not different from the six-month-old, three-month-old, and one-year-old queen bees with three brood combs. However the three months old queen bee with five combs was significantly different from the three combs in all ages. The difference of age of old queen bee was caused by a higher number brood combs, which expanded the cumulative brood combs during the blossom season. The three-month-old queen bee could also produce wider cumulative and relatively brooder combs. According to Morse and Hooper (1985), the presence of fresh pollen during the blossom season will spur the production of broods. Consequently, the quality of the cumulative broods during the blossom season of the three-month-old queen bee with five combs was better.

The quality of the cumulative brood depends on the interaction between the age of the queen bee and number of brood combs (Beyene et al., 2018). The three-month-old queen bees with five and three combs produced the same quality of brood. The three-month-old queen bees were capable of producing the widest range of pollen combs, and they produced a better quality of brood. The six-month-old queen bees with the three brood combs still showed the same ability as other treatments.

The quality of the cumulative brood also depended on the number of brood combs. The quality of the brood with five combs was better than three combs. This difference was due to the wider cumulative brood honeycomb size for five combs compared to three combs. Thus it was directly proportional to the quality of the brood produced (Perichon & Bhatta, 2019). The result of this study indicated that there were a higher number of bees that were born in the hive with five combs compared to three combs; brood rearing as a determinant of quality depended on the number of worker bees born in the hive.

The relative brood quality for all of the experimental scenarios depended on the number of brood combs. The relative brood quality in the hive with three brood combs was higher than the hive with five combs (More et al., 2019; Zanni et al., 2018). Nevertheless, in the beginning of the blossom season, the hive with five brood combs was of a higher quality compared to the hive with three combs. During the blossom season, the brooding quality in the hive with three brood combs experienced better performance, which increased the relative brood quality. In addition, the quality of the brood per colony in the hive with three combs was lower. Thus the improvement observed during the blossom season caused an improvement in the relative brood quality. The size of the relative pollen comb with three combs was wider, so all worker bees maximally used the available pollen to meet the needs of the brood. According to Winston (1987) and Snodgrass &

Erickson (1992), as long as the nest is still open, the worker bees will provide food in large quantities. Involving worker bees in brood keeping, as was the case for the hive with three combs, resulted in better quality of the brood.

Conclusion

We conclude that the brood comb size depended on the number of brood combs and the use of a queen excluder. In addition, the brood comb quality depended on the age of the queen and the number of brood combs. We recommend that in order to increase colony honey production during the blossoming season, keepers should use three-month-old queen bees (R3), the number of brood combs should not exceed three and a queen excluder should be used. We also recommend that a bee colony management system should be used based on the above findings.

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