UDC 633; DOI 10.18551/rjoas.2023-01.10

BENGUK BEAN TEMPE FLOUR (MUCUNA PRURIENS L.) AS POLLEN SUBSTITUTE FOR COLONY PRODUCTIVITY OF APIS MELLIFERA L.

Pinandita Eggi Pur*, Student of Master of Animal Science Program
Faculty of Animal Husbandry, University of Brawijaya, Malang, Indonesia

Junus Mochammad, **Minarti Sri**
Department of Animal Husbandry, Faculty of Animal Husbandry, University of Brawijaya, Malang, Indonesia

*E-mail: pakmakinpps@gmail.com

ABSTRACT
This study aims to analyze and evaluate the feed preferences of Apis mellifera bees to benguk bean tempe flour (Mucuna pruriens L.) as a substitute for pollen. The ingredients used are corn pollen, benguk bean tempe flour (Mucuna pruriens L.), water, bee feed and honey. The method used is experimentally by feeding the bees as much as 300 g every 3 days. There were 4 treatments and each treatment was repeated 5 times. The variables observed were the area of the tiller comb and the weight of the Apis mellifera honey bee colony. The data obtained were tested for assumptions to see the results of observations of homogeneous, additive, and independent normal distribution. The results showed that the effect of substitute feed had a very significant effect ($P < 0.01$) on colony weight with the highest treatment P3 (1.36 ± 0.83c), egg comb area with the highest treatment P3 (790.67 ± 32.55d). , the larval comb area with the highest treatment was P3 (655.92 ± 18.76d), the pupa comb area with the highest treatment was P3 (561.02±21.77d). The conclusion of this study was that the provision of benguk tempeh flour (Mucuna pruriens L) as a substitute for pollen (P3 = 85% Benguk Tempe Flour + 15% honey) gave the best results on the area of tiller combs and the weight of the honeybee colony Apis mellifera L.

KEY WORDS
Honey bee, tiller productivity, colony weight, benguk bean tempe flour.

Apis mellifera honey bee is widely cultivated in Indonesia because it is able to adapt to the environment. Honey bee cultivation follows the spring season as a source of feed which will produce honey based on flower sources. Honey bees feed on nectar and pollen. Bees need food for the development and growth of their colonies. Bees in the process of looking for food use the sun as a reference. The rainy season will cause the length of sunlight to be reduced, so honey bees will find it difficult to find natural food.

The rainy season causes the availability of nectar and pollen to be very less because the pollen will be wet, the nectar is damaged and the bees find it difficult to find food. Lack of feed in bee colonies will inhibit the development of honey bees and will have an impact on decreasing production and colony productivity. Tier nest comb is a comb that functions for the growth and development of prospective bees from the egg, larva and pupa stages. Combing nests of tillers will accelerate their growth if feed requirements are met, while feed requirements that are not fulfilled will interfere with the growth of tillers.

Provision of pollen substitute feed is an alternative to maintain colony production and productivity in the rainy season. Farmers can take advantage of locally available ingredients and have the potential as a substitute feed source. Local ingredients that have about 20-40% protein such as beans can be recommended as a substitute for pollen. The advantage of using ingredients from nuts is that they are relatively cheap and easy to obtain. One type of local beans that has the potential to be used as a substitute for pollen is benguk beans. Benguk (Mucuna pruriens L.) has a fairly high production of up to 0.51 tons per hectare.

Benguk beans producing areas are centered in Java, especially those with dry agricultural areas such as East Java, Central Java and Yogyakarta (Amanah et al., 2019).

Beans (Mucuna pruriens L.) have potential as a source of protein but still have some weaknesses such as toxic and anti-nutritional properties. Beans (Mucuna pruriens L.) have anti-nutritional compounds that can cause stunted bee growth because it interferes with metabolic processes and nutrient absorption. Anti-nutrients in benguk beans (Mucuna pruriens L.) are phytic acid and toxic properties are hydrogen cyanide. One way to reduce levels of anti-nutritional compounds is by using a good and correct process to ferment benguk beans (Mucuna pruriens L.) into tempeh. During the fermentation process, tempeh molds produce proteolytic enzymes that break down proteins into amino acids so that dissolved nitrogen increases. Based on this, benguk bean (Mucuna pruriens L.) has the potential as a substitute for pollen for bees. One alternative to processing tempeh products that extends shelf life and use is made of tempeh flour. (Widowati, 2013) stated that the recommended replacement pollen particle size is below 500 m so that the feed can fit into the bee's mouth. Tempe flour whose size and nutritional content resembles natural pollen can increase the productivity of honey bees.

MATERIALS AND METHODS OF RESEARCH

This research was conducted in February – March 2022 at PT. Kembang Joyo is located in Karangploso, Malang. The proximate analysis of the feed that will be given to the bees is carried out at the Laboratory of Nutrition and Animal Feed, Faculty of Animal Husbandry, University of Brawijaya. The material used is honey bee (Apis mellifera L.) as many as 20 colony boxes (stup) with 6-7 combs. The colony box contains eggs, larvae, pupae, queen bees, worker bees, male bees and feed. Colonies are sought to have uniformity in the age of the queen, the age of the tillers (larvae, eggs and pupae) and the number of tillers (larvae, eggs and pupae). The colonies used belonged to the PT. Joyo Flower. Benguk (Mucuna pruriens L.) was obtained from the Yogyakarta area. Pollen substitute feed comes from benguk bean tempe flour (Mucuna pruriens L) with a size below 500 m (Widowati, 2013).

The method used is experimentally by feeding the bees as much as 300 g every 3 days. There were 4 treatments and each treatment was repeated 5 times. The following treatments will be given to livestock, namely P1: 100% Pollen (Control Treatment), P2: 85 % Benguk Bean Tempe Flour + 15 % Water, P23: 85 % Benguk Bean Tempe Flour + 15 % Honey, P4: Benguk Bean Tempe Flour 85% + Bee Feed 15%. The equipment used for beekeeping includes a colony box with a size of p x l x t (40x30x20), clear plastic (mica), 3 colored markers, analytical scales, combs, frames, feed containers, fumes, and bee veils.

The measurement of the area of the tillers comb (Eggs, Larvae, and Pupae):

1. Egg Comb Area. The measurement of the area of egg production is done by attaching transparent plastic to the comb of the nest, then drawing it with a marker. The transparent plastic that has a wide picture of the sample nest comb is photocopied. Weigh the weight of the copy paper (b) g and measure the area of the photocopy paper. Cut the photocopy paper of the irregular area of the egg comb and weigh it, then convert the results to determine the area of the egg comb. Then coded T1 on the first observation, T2 on the second observation and T3 on the third observation and so on. Observation of the comb area of eggs was carried out once every 3 days for 9 days.

2. Larvae comb area. Measurement of the area of larval production was carried out by attaching transparent plastic to the comb of the nest, then drawing it with a marker. The transparent plastic that has a wide picture of the sample nest comb is photocopied. Weigh the weight of the copy paper (b) g and measure the area of the photocopy paper. Cut the photocopy paper of the irregular area of the larval comb and weigh it, then convert the results to determine the area of the larval comb. Then coded L1 on the first observation, L2 on the second observation and L3 on the third observation and so on. Observation of the area of the tiller comb was carried out once every 10 days for 30 days.

3. Pupa Comb Area. Measurement of the area of pupa production was carried out by attaching transparent plastic to the comb of the nest, then drawing it with a marker. The transparent plastic that has a wide picture of the sample nest comb is photocopied. Weigh the weight of the copy paper (b) g and measure the area of the photocopy paper. Cut the photocopy paper of the irregular area of the pupa comb and weigh it, then convert the results to determine the area of the pupa comb. Then coded P1 on the first observation, P2 on the second observation and P3 on the third observation and so on. Observation of the area of the tiller comb was carried out once every 8 days for 24 days.

4. Colony Weight. The method of collecting data on colony weight gain (Bs) is to weigh the stup containing bees. After that the bees were transferred to another box, the stup was weighed without the bees to obtain the empty stup weight (Bk). The difference between Bs and Bk is the total weight of the bees (Bt). Then coded B1 for the first observation, B2 for the second observation and B3 for the third observation and so on. Colony weight observations were carried out once a week for 1 month.

The observed variables are as follows:

$$\text{Egg comb area: (C g)/(b g) x a cm}^2 = \text{d cm}^2$$

Where:

- a: area of photocopy paper (cm^2);
- b: Weight of photocopy paper (gr);
- c: Weight of combed drawing paper containing eggs (gr);
- d: egg comb area (cm^2).

$$\text{Larva comb area: (C g)/(b g) x a cm}^2 = \text{d cm}^2$$

Where:

- a: area of photocopy paper (cm^2);
- b: Weight of photocopy paper (gr);
- c: Weight of combed drawing paper containing larvae (gr);
- d: larval comb area (cm^2).

$$\text{Pupa comb area: (C g)/(b g) x a cm2} = \text{d cm2}$$

Where:

- a: area of photocopy paper (cm^2);
- b: Weight of photocopy paper (gr);
- c: Weight of combed cut paper containing pupae (gr);
- d: the area of the comb of the pupa (cm^2).

$$\text{Colony weight: Bt} = \text{Bs} - \text{Bk}$$

Where:

- Bt: Total weight of bees;
- Bs: Weight of bee colony;
- Bk: Weight of empty colony box.

The data obtained were tested for assumptions to see the results of observations of homogeneous, additive, and independent normal distribution. If it is homogeneous and additive, it can be said that the distribution is normal and independent (DNI) then parametric test is performed with Completely Randomized Design (CRD).

The following formula is from RAL (Kusriningrum, 2008):

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Where:

- Y_{ij} = observation value in the 1st - 6th treatment and 1st - 4th repetitions;
- μ = general average;

- τ_l = effect of treatment 1-6;
- ε_{ij} = effect of experimental error on treatments 1-6 and replications 1-4.

After being analyzed using RAL if the results are significantly different, then proceed with the orthogonal polynomial test (Gomez & Arturo, 2015):

$$Y = \alpha + \beta_1 X + \beta_2 X^2 + \ldots + \beta_n X^n$$

Where:

- α: intercept;
- β: partial regression coefficient related to the degree of polynomial 1-24.

RESULTS AND DISCUSSION

The results of the research were homogeneity test and additivity test for egg, larva and pupae comb area data. The test results indicate that the data are homogeneous and additive, after the assumption test is met, then analysis of variance is carried out. The average Tillers Comb Area results are listed in Table 1.

Table 1 – Average Tillers Comb Area

Treatment	Egg Comb Area	Larva Comb Area	Pupa Comb Area
P_1	772,56±50,01^c	642,62 ± 27,92^c	558,18±48,89^c
P_2	505,33±33,91^a	422,73 ± 33,89^a	329,22±40,94^a
P_3	790,67±32,55^d	655,92 ± 18,76^d	561,02±21,77^d
P_4	687,43±20,02^b	533,12 ± 44,80^b	455,45±37,94^b

Note: Different superscripts showed a very significant difference (P<0.01).

The results of the analysis of variance showed that the use of Benguk Bean Tempe flour (Mucuna pruriens L) had a very significant effect (P<0.01) on the egg comb area. The treatment at P3 was the combed area of the egg nest with the highest average of (790.67±32.55 d) while the lowest egg-combing area was in treatment P2 with an average of (505.33±33.91 a). It can be seen that the additional feed in the form of bean flour has an effect on the comb area of the egg nest. The P3 treatment showed the highest results because the bees were thought to be favored by the bees that had a percentage of bean bean tempeh as much as 85% with a mixture of 15% honey, while the P2 treatment had a low average because the bees did not really like the additional feed in the form of benguk beans which had a percentage of tempeh. bean benguk as much as 85% with a mixture of 15% water, worker bees need high protein to be given to the queen bee, because to produce good eggs, if the eggs produced are many, the nest will be wider. This is in accordance with the opinion of (Hoan & Hoan, 2021) which states that the adequacy of the food received will determine the production of eggs produced and is supported by the statement of (Wegener et al., 2009) that when the availability of feed with high nutrient content is abundant, the hatching rate will increase. higher eggs.

How to maintain egg life, worker bees provide feed with high protein, comparable to the statement by (Mayaut et al., 2020) that the speed of bee metamorphosis can be determined from the availability of feed around the hive. The faster the metamorphosis, the larger the bee colony and the higher the resulting production. The success rate of bee eggs to hatch into larvae to pupae is influenced by several factors, namely the presence of feed sources and worker bee cannibalism caused by a lack of protein supply in the colony (Khoury et al., 2013).

The results of the analysis of variance showed that the provision of benguk bean tempe flour (Mucuna pruriens L) had a very significant effect (P<0.01) on the comb area of the larvae. The treatment at P3 was the comb area of the larval nest with the highest average of 655.92 ± 18.76d, while the lowest combed area of the larval nest was in the P2 treatment with an average of 422.73 ± 33.89a. It can be seen that P3 with the addition of bean curd flour as much as 85% has an effect on the comb area of the larval nest. This shows that the

need for pollen can be available which will support the growth and development of larvae. If the need for pollen is limited it will affect the growth of the larva itself. To support the availability of pollen during the famine season, the bees are given additional feed in the form of bean flour which has high protein.

According to (Collins, 2004) which states that the eggs hatch into larvae after a few days of being fed by worker bees. Larvae must consume feed with nutritional content and very large amounts to support their growth rate. The number of larvae is influenced by the feed given in the nest. This is supported by the statement of (Ghosh et al., 2020) that larval development requires feed with sufficient nutritional value. The feed that bees need contains essential amino acids, high protein, relatively little amount of fat and rich in minerals. The nutritional needs of feed in the development phase of honey bees from larvae to adult bees such as decreasing the amount of carbohydrates, increasing protein and ash levels. All phases of honey bees, especially the stage of becoming an adult bee, really need a high source of protein (Ghosh et al., 2016). The statement of (Heimkem et al., 2009) adds that larvae that are still lacking in feed and larvae that are sufficient to feed will emit odor signals to inform nurse bees.

The results of the analysis of variance showed that the provision of benguk bean tempe flour (Mucuna pruriens L) had a very significant effect (P<0.01) on the area of the pupa comb. Based on Table 19, it can be seen that in treatment P3 is the width of the pupa nest comb with the highest average of 561.02±21.77d. While the P2 treatment is the area of the pupa nest comb with the lowest average of 558.18 ± 48.89. This is P3 treatment with the addition of benguk bean flour (Mucuna pruriens L.) as much as 85% with a mixture of 15% honey affects the increase in the area of comb pupae because the feed requirements provided are met and used for the growth period of the pupae. Pupae that do not develop may be caused during the previous phase of poor treatment, due to feed and environmental factors. (Kuntadi, 2008) added that the bee colony was weakened due to high rainfall, causing the bees to find it difficult to get food, so this caused the pupa comb area to narrow and not develop optimally.

The need for nutritional content in feed differs between stages of development of worker bees. This is in line with the statement of (Ghosh et al., 2021) that the total requirement for amino acid content differs between developmental stages. The requirement for the total amount of amino acids increased when the prepupa turned into a pupa even though the weight was reduced due to the higher prepupa water content. The development of muscles and other tissues during the pupal stage requires an increase in the protein content available from the feed.

The scarcity of pollen sources is an obstacle in meeting the feed needs of honeybees, so the development of local pollen substitution is very important for Apis mellifera. Limited food sources often occur during the dry season, replacement feed during this period is very necessary. Efforts to develop a pollen substitute are using materials that are relatively inexpensive, have high nutritional value and are easily available. This study used bean (Mucuna pruriens L.) as a substitute for pollen. Processing of benguk bean (Mucuna pruriens L.) as flour by immersion and fermentation treatment which aims to remove anti-nutritional compounds. The data from the research were carried out by homogeneity test and additivity test for colony weight data. The test results indicate that the data are homogeneous and additive, after the assumption test is met, then analysis of variance is carried out. The average colony weight results resulting from the first phase of the study are listed in Table 2.

Table 2 – Average Colony Weight

Treatment	Colony Weight (Kg)
P_1	$1,33 \pm 1,03^b$
P_2	$1,31 \pm 0,79^a$
P_3	$1,31 \pm 0,79^a$
P_4	$1,36 \pm 0,83^c$

Notes: Different superscripts showed a very significant difference (P<0.

The results of the analysis of variance showed that the provision of benguk bean tempe flour (Mucuna pruriens L) had a very significant effect (P<0.01) on the weight of the bee colony. Treatment on P3 was the colony weight with the highest mean of 1.36 ± 0.83c while the lowest weight was on treatment P2 with an average of (1.31 ± 0.79a) and P4 with an average of (1.31 ± 0.79a). It can be seen that the additional feed in the form of bean bean flour has an effect on colony weight. Treatment P4 showed the highest results because it was suspected that the feed provided was favored by bees which had a percentage of bean benguk tempeh as much as 85% with a mixture of 15% honey, while treatments P2 and P4 had a low average because the bees did not really like additional feed in the form of benguk beans which had a percentage of Bean curd tempeh as much as 85% with a mixture of water and bee feed. This can be seen during the study of substitute feed with 85% bean flour with a mixture of 15% honey which was often visited by worker bees. It is possible that the bee colony has not received sufficient supply of pollen available in nature, so that the substitute feed provided makes a difference to the colony's body weight gain, but the treatment of natural pollen with bean flour as a substitute for pollen is better than the provision of natural pollen. This could be due to the substitute for pollen, tempeh, benguk bean (Mucuna pruriens L.) which had undergone processing and received the addition of honey.

Organoleptically, the aroma of honey has a distinctive odor that allows it to attract the attention of worker bees, in contrast to the provision of bean bean flour with a mixture of water and bee feed which tends to be less favored by worker bees. It is interesting that the attention of worker bees will increase daily visits to pollen, thereby allowing consumption of pollen as a substitute for bean curd which has an impact on increasing the weight of the honey bee colony.

Based on previous studies, it was shown that there is no substitute feed whose quality is equal to natural feed (Irandoust & Ebadi, 2013). Although some rations have a higher protein content than natural pollen, bees are more attracted to fresh natural pollen than to substitute rations. This is different from this study where the natural pollen treatment feed was not provided in fresh form but natural pollen that had been dried and had been stored for a certain period of time, so that this natural pollen was less favored by bees. The fraction of the attractant contained in natural pollen is not owned by the substitute ration. This is in accordance with the statement of (Naik et al., 2005) that honey bees will visit surrounding flowers, if the flowers contain attractant compounds and vice versa if the flowers contain repellent compounds, honey bees will avoid them. According to (Bayram et al., 2021) stated that the content of phenolic compounds can attract the attention of bees. The content of phenol in pollen can be related to the season when the pollen was collected and the origin of the plant, which indicates that pollen containing phenolic compounds can be affected by temperature and storage time. The results of this study indicate that the substitution of pollen from bean flour can be said to be ideal.

The period of time between March and May is the time when pollen is hard to find in nature. The provision of pollen substitutes at that time was very appropriate to do so that the growth of the bee colony was not disturbed by a lack of feed. This increase in colony weight was thought to be caused by an increase in the area of chicks, larvae and pupae. Worker bee activity is more devoted to care and feeding for larvae.

CONCLUSION

The provision of benguk bean tempe flour (Mucuna pruriens L) with a mixture of honey with 85% bean curd tempe flour (Mucuna pruriens L) and 15% honey resulted in better colony performance, range of tillers and colony weight.

ACKNOWLEDGMENTS

Authors express gratitude to:

- Rector of University of Brawijaya who has funded the research;
- Dean of the Faculty of Animal Science, University of Brawijaya, who gave approval and support for facilities through the Professor's Grant research program;

- PT Kembang Joyo which has provided colonies and other research facilities;
- All parties involved and participating in the research.

REFERENCES

1. Amanah, Y. S., Sya'di, Y. K., & Handarsari, E. (2019). Kadar Protein dan Tekstur pada Tempe Koro Benguk dengan Substitusi Kedelai Hitam. Pangan Dan Gizi, 9(2), 119–127.
2. Bayram, N. E., Gercek, Y. C., Celik, C., Mayda, N., Kostic, A. Z., Dramicanin, A. M., & Ozkok, A. (2021). Phenolic and Free Amino Acid Profiles of Bee Bread and Bee Pollen with the Same Botanical Origin Similarities and Differences. Arabian Journal of Chemistry, 14, 1–12.
3. Collins, A. M. (2004). Variation in Time of Egg Hatch by the Honey Bee, Apis mellifera (Hymenoptera: Apidae). Annals of the Entomological Society of America, 97(1), 139–146.
4. Ghosh, S., Herren, P., Rochow, V. B. M., & Jung, C. (2021). Nutritional Composition of Honey Bee Drones of Two Subspecies Relative to Their Pupal Developmental Stages. Insect, 12(759), 1–15.
5. Ghosh, S., Jung, C., & Rochow, V. B. M. (2016). Nutritional Value and Chemical Composition of Larvae, Pupae, and Adults of Worker Honey Bee, Apis Mellifera Ligustica as A Sustainable Food Source. Asia-Pacific Entomology, 19, 487–495.
6. Ghosh, S., Sohn, H. Y., Pyo, S. J., Jensen, A. B., Rochow, V. B. M., & Jung, C. (2020). Nutritional Composition of Apis mellifera Drones from Korea and Denmark as a Potential Sustainable Alternative Food Source: Comparison Between Developmental Stages. Foods, 9(389), 1–16.
7. Gomez, K. A., & Arturo, A. G. (2015). Prosedur Statistik untuk Penelitian Pertanian. UI Press.
8. Heimkem, C., Aumeier, P., & Kirchner, W. H. (2009). Mechanisms of Food Provisioning of Honeybee Larvae by Worker Bees. Experimental Biology, 212, 1032–1035.
9. Hoan, N., & Hoan, P. D. (2021). Factor Effects on Number of Eggs Laid of Queen Bee Species Apis Cerena at Northeastern Region of Vietnam. Livestock Research for Rural Development, 33(8), 1–9.
10. Irandoust, H., & Ebadi, R. (2013). Nutritional Effects of High Protein Feeds on Growth, Development, Performance and Overwintering of Honey Bee (Apis Mellifera L.). International Journal of Advanced Biological and Biomedical Research, 1(6), 601–613.
11. Khoury, D. S., Barron, A. B., & Myerscough, M. R. (2013). Modeling Food and Population Dynamics in Honey Bee Colonies. PLOS One, 8(5), 1–7.
12. Kuntadi. (2008). Perkembangan Koloni Apis mellifera L. yang diberi Tiga Formula Kedelai sebagai Pakan Buatan Pengganti Serbuksari. Jurnal Penelitian Hutan Dan Konversi Alam, 5(4), 2–3.
13. Kusriningrum, R. S. (2008). Buku Ajar Perancangan Percobaan, Fakultas kedokteran Hewan Universitas Airlangga. Dani Abadi.
14. Mayaut, G., Nindatu, M., & de Kock, R. H. (2020). Beda Waktu Metamorfosis Lebah Madu Apis mellifera di Pulau Romang. Journal Rumphius Pattimura Biological, 2(1), 16–23.
15. Naik, D. G., Dandge, C., Puntambekar, H., & Patil, T. (2005). Attractant and Repellent Properties of Swertia Densifolia Leaf Extract Towards Apis cerana indica. Journal of Apicultural Research, 44(3), 116–118.
16. Wegener, J., Lorens, M. W., & Bienefeld, K. (2009). Differences between Queen- and Worker-Laid Male Eggs of the Honey Bee (Apis mellifera). Apidologie, 41, 116–126.
17. Widowati. (2013). Pollen Substitute Pengganti Serbuk Sari Alami Bagi Lebah Madu. Journal WIDYA Kesehatan Dan Lingkungan, 1(1), 31–36.