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Research Article

Impact of Yeast Diet on the Number of Eggs and Larvae Produced in Honey Bee Colonies (*Apis Mellifera* L.) Apidae: Hymenoptera

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Abstract: Honey bees (*Apis mellifera* L.) are important social insects because of the honey production and pollination services they provide. Diet quality affects bee progression through different life stages, adult longevity, fecundity and foraging activity, among other likely phenotypes. This study was conducted to determine the probable effect of colony food availability on the number of eggs and resulting larvae produced by honey bee colonies. Sixteen honey bee hives were used in the study. The hives were split into groups of four, with each group receiving one of the following four treatment diets: (1) T1 – sugar water (1 l water + 250 g sugar), (2) T2 - yeast water (1 l water and 50 g Brewer's yeast–non-floral protein diet in dry form), (3) T3 – water (1 l water), and (4) T4 – no diet. The impact of the colony diet (sugar syrup and yeast with treatments mentioned above) on the number of eggs and larvae produced was determined using a one-way ANOVA conducted using the statistical program "R" version 2.15.3. Where appropriate, means were compared using the least significant difference (LSD). Numerically, the average number of eggs and larvae on sugar solution were 24.20 ± 1.72 and 26.8 ± 1.808 respectively, while on the yeast diet were 33.66 ± 2.92 and 31.55 ± 2.324, respectively. Significantly, the number of eggs (P-value 4.74E-10, F value 21.50528 and F-tabulated value as 2.731807) and larvae (P-value 5.31E-05, F-value 8.70 and F-tabulated value 2.73) produced was significant when colonies were fed with yeast and sugar solution.

Keywords: Larval Duration, Eggs, Honey Bees, Artificial Diet.

1. INTRODUCTION

Proteins, lipids, carbohydrates, vitamins, other minerals, and water are present in plant nectar [1]. Western honey bees (*Apis mellifera* L.) collect nectar and convert it to honey. Honey serves as the bees' carbohydrate source but also provides other essential elements and compounds. Honey bees use these elements in the scarcity of flora and fauna [2]. Honey bees also feed on bee bread; a food resource produced from pollen [3]. Adult honey bees feed directly on these foodstuffs. However, immature honey bees feed on the glandular secretions provided to them by their adult nurse worker sisters, though some nectar and pollen may be added to the larval diet [4]. The quality of the food supplies available in the nest impacts nurse production of the larval diet [5]. The lack of quality food resources causes the nurse honey bees to use the protein and lipid reserved in their own bodies to produce larval food and also for their survival during periods of dearth [2, 6].

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However, the brood growth on alternate pollen food and or supplemental diet in the bee hives is observed as less comparative to the fresh natural pollen [7]. Although the supplemental diet which contains yeast was consumed in surplus comparatively because of their constant behavior and cravings [8] but the fecundity was not in significant difference with the natural and fresh pollen. In the spring season, an increase in the honey bee brood development was observed at the supplemental pollen mixed diet [9]. The provision of a supplemental protein diet mixed with brood pheromones showed an increase in brood and adult bees' strength compared to the colonies with only a protein diet [10].

In the late spring or early winter, the beekeepers supply food to the colonies to encourage brood rearing and or nutritional strain in the dearth periods to cope with nutritious feeding [11]. The physiology and behavior of adult bees can be affected by the quality of the diet they receive [6]. Worker bees provide 25 - 37.5 mg of protein in the diet of each larva [12], some of this deriving from bee bread [13, 14]. In the first three days of larval growth, the sugar content of the larval diet (fructose and sucrose) is about 18%, but it increases to 45% in the final two days of larval development. The honey bees which efficiently consume the supplemental diet in a surplus of 23% of protein could have better potential of rearing larvae [15].

Honey bees that consumed diet with more than 23% protein could rear honey bee larvae. A larval diet encompassing the sugars and dry yeast in the bee hives [16] increases longevity and further can be boosted by raising the amount of glucose, yeast, and fructose in their diet [17]. During the larval developmental period, a larva is regularly observed and little by little nourished about 135−143 times on the food preserved by the worker bees [16, 18]. The supplemental diet enriched with carbohydrates and proteins is consumed more by the bee larvae, so as per a rough appraisal, the entire proteins and carbohydrates for the growth and development consumed by one larva are 25−37.5 mg and 59.4 mg respectively [12].

For proper honey bee colonies management, beekeepers should have the knowledge of different apiculture management practices in depth [19]. Beekeepers often supplement the honey bee diet by feeding colonies a carbohydrate source (usually sugar or corn syrup) and a protein source (typically a pollen patty or supplement). However, the success of these diets often is determined as a colony's consumption of the diet rather than the colony's effective utilization of it. A high-quality food substitute should contain ingredients that supply the essential colony growth nutrients and nutrients for individual bee development, longevity, and productivity.

It is experiential that even if the principles of migratory beekeeping are followed, around 40% of bee hives decomposed through the dearth (scarcity of flora and food resources) time of the year. The stipulation of the supplementary artificial diet to the colonies has unanimously been considered and administered for the brood rearing egg laying, as well as for the foraging activities that possibly can uphold all the colony parameters. A numerous supplemental diet formulations have systematically been developed through combination of various components and observed by a diverse population of workers around the globe regarding commercial beekeeping point of view [20−25].

The primary components of yeast are polysaccharides such mannans, chitins, and glucans. The benefits from yeasts appear to include the production of vitamins that can enhance bee food [26]. Additionally, yeasts were found in large quantities in newly emerging bees and nurse bees, and it has been suggested that they aid in the digestion of pollen and the production of royal jelly [27]. Herein, we determined the impact of feeding colonies with sugar water fortified with yeast on the number of eggs and larvae produced in treated colonies. We expected the increased number of eggs in the cells and larvae in the colony.

2. MATERIALS AND METHODS

This experiment was conducted in the apiary of the College of Agriculture, University of Sargodha located at 32.08° N, 72.67° E and 193 m above sea level. The temperature in the study area was 22.8 °C to a warm 31.9 °C under the sub-tropical, semi-arid and clear climatic conditions. Honey bee colonies which were Langstroth equipped and single deep manufactured containing ten frames per colony were established.

The colonies were equalized regarding the larval strength, empty egg cells, and honey stores before the experiment and exposed to comparable food resources (daily foraging activities of bees and food available in the hive) as well and further placed on iron stands ~0.3 m high to reduce the other contaminants' effects. There was a 200 meters distance among each experimental colony. The colonies were checked for *varroa destructor*. Since the adult mite infested the honey bees, so a close inspection of the drone brood strength was done to check its infestation, however chemical treatment was applied. The experimental colonies were critically checked for diseases (discoloring of sealed brood, brood combs, scattering of sealed brood, punctured cell capping, sunken cells) in order to avoid any sort of error in the experiment.

Sixteen honey bee colonies were split into four groups of four colonies/group. The groups were assigned one of the following diet regimens: (1) T1 – sugar water (1:1 water + 250 g sugar), (2) T2 – yeast water (1:1 water and 50 g Brewers' yeast; non-floral protein diet for honey bees), (3) T3 – water (1:1 water), and (4) T4 – no diet. The varying diets were provided to the colonies in polythene bags (measuring approximately 2 × 10 ×15 cm), placing them beside the frames.

Twenty randomly chosen, empty cells in the brood nest were monitored on each of six frames for all 16 colonies (120 cells per colony) twice daily (09:00 and 16:00) once the diets were provided to the colonies. The cells were physically marked on each frame with the help of permanent marker. During the monitoring period, we recorded the day that the queen laid an egg in each cell, the day the cells were capped, and the day the resulting adult bees emerged from the cells. Mostly, the larvae were counted through physical observation especially when they were 0-24 hours old. These data allowed us to calculate the length of each developmental period. The impact of the colony diet on the number of eggs, larvae, and adults was determined using a one-way ANOVA conducted using the statistical program "R" version 2.15.3. Where appropriate, means were compared using LSD.

3. RESULTS

3.1 Effect of Diets on Eggs

The effect of different diets on several eggs is presented in Figure 1. The sugar solution diet increased the number of eggs at a rapid rate in the first 10 days, then the increase in number of eggs started to decrease. With a maximum average of 47.55 ± 1.55 and a minimum average of 15.8 ± 1.58, the average number of eggs in the sugar solution was 24.20 ± 1.72. Results showed that a sugar diet has a significant effect on the number of eggs in the colony. There was an excellent increase in the number of eggs in the first 10 days on the yeast diet then the population gradually started decreasing

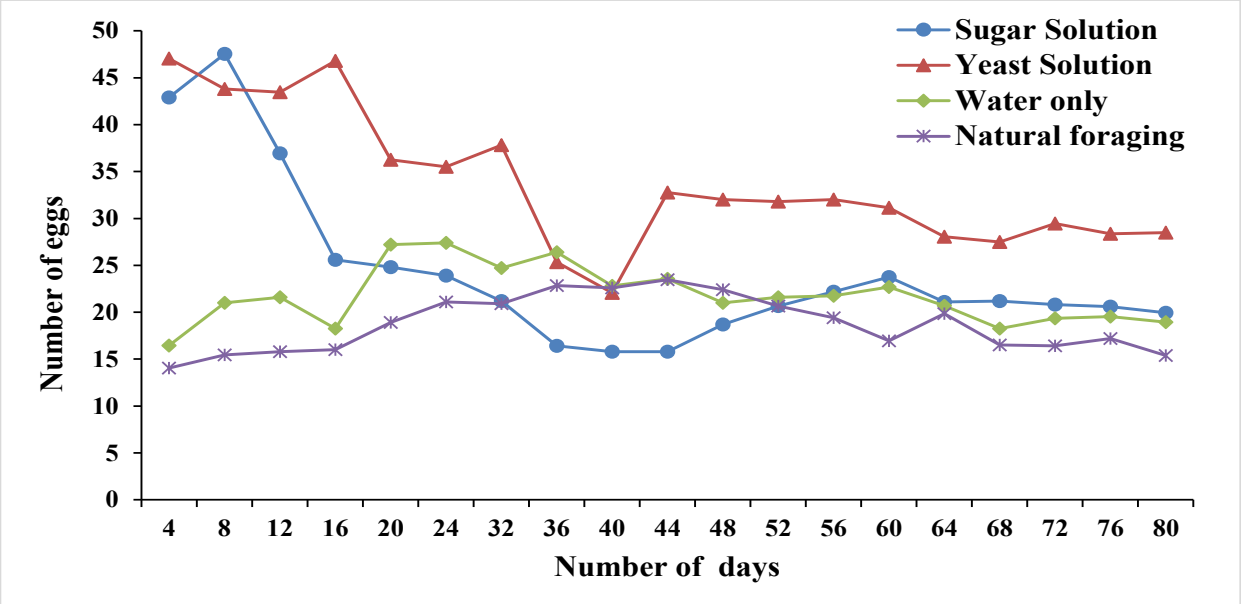

Fig. 1. Effect of varying artificial diets on the number of eggs of honey bees *Apis mellifera.*

throughout. The average number of eggs consumed on this diet was 33.66 ± 2.92, with a maximum average of 47.05 ± 7.94 and a minimum average of 22.05 ± 1.44. The yeast diet has a significant impact on the number of eggs produced in the colony.

The average number of eggs on the water diet was 21.74 ± 1.74 with the highest average of 27.4 ± 2.67 and a minimum value of 16.45 ± 1.29. The results showed that water treatment had a positive effect, but it was modest compared to the other three nutritional parameters and helped increase egg numbers. By natural flora and fauna, the number of eggs increased gradually as well as continuously. This is because the only natural source of protein is pollen for bees. The mean number of eggs in natural foraging was 18.72 ± 1.12, with a highest value of 23.45 ± 1.23 and a minimum of 14.05 ± 0.76. The results showed that natural foraging gave strength to the colony and contained a large number of eggs compared to other parameters.

3.2 Analysis of Variance

ANOVA was piloted to assess the influence of the different diets on the number of eggs in the treated colonies. The ANOVA result showed the significant effect of diet with the F crit value as 2.731807, P-value 4.74E-10, and F value 21.50528 (Table 1). The maximum egg population was 47.55 and the minimum population was 15.4, on 15.03.2014.

3.3 LSD Test for Means Comparison

After finding significant results from the ANOVA, a Post hoc test LSD was used to compare the difference between all diet parameters (Table 2).

The model's findings demonstrated that yeast solution has a greater impact on egg development than any other diet, with an average mean of 33.66 ± 7.22 value for St. error (significant letter "a"). With an average mean of 21.75 and a St. error of 3.08 (significant letter "bc"), the water diet was then found to be effective. The sugar solution with an average mean of 24.20 ± 8.75 (significant letter "b") was discovered to be the best. The average mean and standard error for natural foraging are 18.73 and 2.97, respectively (significant later "c").

3.4 Effect of Diets on Larval Population

On the first day of data collection, due to low sugar content, such as fructose and sucrose in the brood food, which is only about 18% during the first three days of larval development before rising to 45% during the final days of development, the average number of larvae was "0.8" (standard error 0.2). Due to continuous feeding, population growth began after three days and continued for the next 20 days. Following that, the strength of the larvae decreased slightly for the first 14 days before steadily increasing for the following 16 days until the end.

In our experiments, the yeast solution contributed significantly to the development of larval strength compared to other nutritional parameters. The mean number of larval bees on yeast was 20.04, with a maximum mean of 26.8 and a minimum mean of 0.8. A sugar solution

Table 1. Analysis of variance for the number of eggs.

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	2373.188553	3	791.0628509	21.50527808	4.74E-10	2.731807
Within groups	2648.490526	72	36.78459064			
Total	5021.679079	75				

Table 2. Comparative effects of varying artificial honey bee diet levels on the number of eggs of *Apis mellifera.*

Treatments	Means	St. Error	Significant letters
Yeast solution	33.66	7.22	a
Water only	21.75	3.08	b
Sugar solution	24.20	8.75	bc
Natural foraging	18.73	2.97	c

diet showed a significant difference in larval development as well. On the yeast diet, there was population fluctuation since the increase in larval population was normal in the first 10 days and then it increased at a higher rate from the 11th to14th day and then decreased slowly in the next 11 days. The mean number of sucrose-fed larvae was 23.93, along with a maximum mean of 31.55 and a minimum mean of 1.8.

When watered, populations were low for the first 14 days, then increased for up to 26 days, then decreased for 5–7 days, and then increased again, resulting in increased larval numbers. The average number of larvae at watering was 22.48, with a maximum mean of 32.65 (standard error ± 3.43) and a minimum mean of 0.45 (standard error ± 0.11). Results revealed that water is of great importance for the survival of the larvae. Larval numbers slowly increased as worker bees visited and accessed natural flora and fauna while naturally searching for food (pollen/nectar). The mean larval number that was recorded on this diet was 18.13, with a maximum mean of 26.05 (standard error ± 3.68) and a minimum mean of 0.95 (standard error ± 0.19). The results showed that natural foraging by worker bees to feed larvae is crucial for the development of larval numbers within the colony.

When fed the sucrose solution, the maximum mean number of larvae in worker bees was noted on day 55 of feeding and was 26.8 (standard error ± 1.80), with a minimum mean value of 0.8 (standard error ± 0.2) on the first day of data collection. On the 14th day of data collection for the yeast solution, the maximum mean worker bee larvae were recorded, coming in at 31.55 (standard error ± 2.32), and the minimum mean was recorded, coming in at 1.8 (standard error ± 0.33) (Figure 2).

3.5 Analysis of Variance

An ANOVA was performed to assess the influence of different honey bee diets on honey bee larval numbers. ANOVA showed significant results of diets with a P value of 5.31E-05, an F value of 8.70, and an aggregated F value of 2.73. The highest population was 32.65 and lowest was 0.8 (Table 3).

3.6 LSD Test for Means Comparison

After finding significant results from the ANOVA, a post-hoc LSD test was used to compare differences between all dietary parameters in honey bee larval numbers (Table 4). Dietary differences were compared using the LSD post-hoc test. The LSD results showed the superior effect of yeast solution on the larval population, with a mean of 23.93 and a standard error of 6.34 (significant letter "a") compared to the other three diets. The water-only then followed the yeast solution diet and gave the best results, with a mean of 22.48 and a standard

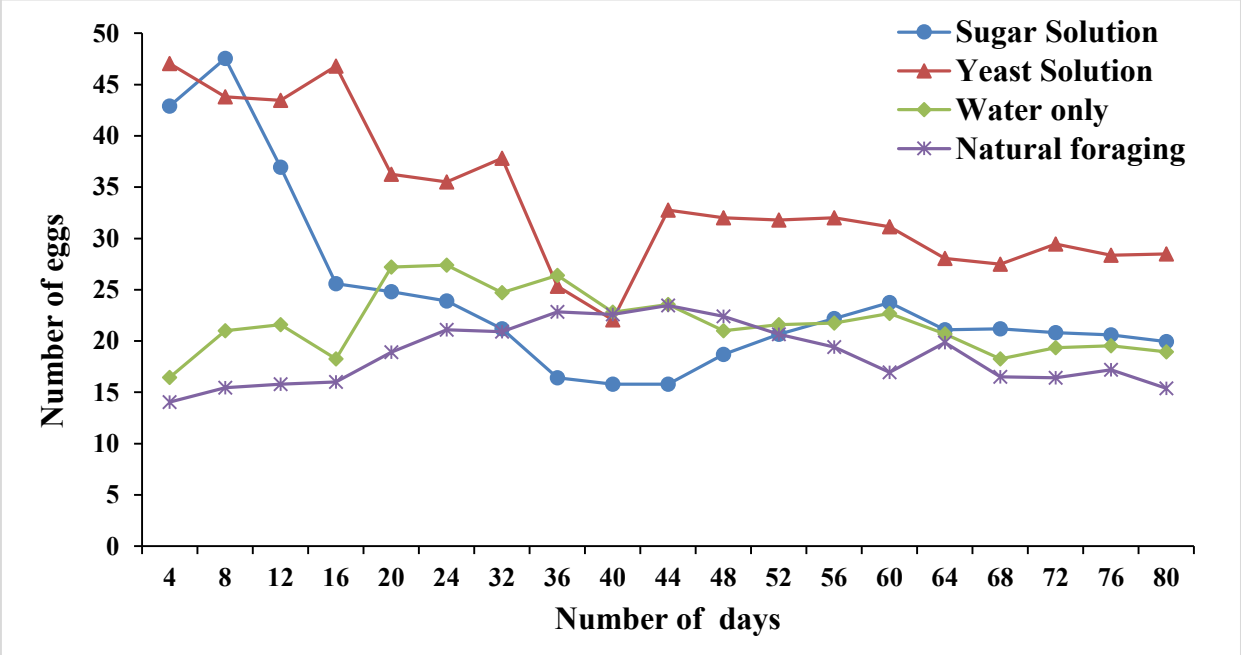

Fig. 2. Effect of varying artificial diets on the number of larvae of honey bees *Apis mellifera.*

Table 3. Analysis of variance for the number of larvae of honey bees.

Source of variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between groups	14.37800817	3	4.79266939	8.70295	<0.000	2.731807037
Within groups	39.65002058	72	0.55069473			
Total	54.02802875	75				

Table 4. Comparative effects of varying artificial honey bee diet levels on the number of larvae of *Apis mellifera.*

Treatments	Means	St. Error	Significant letters
Yeast solution	23.934	6.343	a
Water only	22.489	7.859	a
Sugar solution	20.047	5.503	ab
Natural foraging	18.134	6.036	b

error of 7.85 (significant letter 'a'). The sugar solution then contained a mean of 20.04 with a standard error of 5.50 (significant letters 'ab'), making it a suitable diet for larval development. Ultimately, natural foraging showed lowest effect on larval development. The mean was 18.13 with a standard error of 6.03 for significant 'b'.

4. DISCUSSION

Studies on various dietary parameters associated with worker bee longevity have shown differences in honey bee larval longevity. Four artificial diets (sugar solution, yeast solution, water only, and natural foraging) were used, and these diets showed a significant increase in worker bee lifespan, which had the greatest effect on growth, development, and longevity, and was also favored by larval bees. The maximum average for larvae was 31.55. Many different authors have reported that artificial feeding has positive effects on larval stage dispersal, development, and longevity of honey bees [14]. Steen [28] has explained that the pollen substitutes had a remarkable increase in the life span of honey bees. By frequent or prolonged use of protein-supplemental diets, the bees and castes had negative effects in foraging and other colony activities [29]. However, a lot of scientists reported that supplemental feeding to honey bees can be effective for their growth and development. Yeasts are more attractive to bees for their growth and development because the composition of protein levels is around 50% and normally more fair set of amino acids are also provided by the yeast. Most appropriately yeast is composed of fat 1.0%, 51.8% peptones, proteins, as well as amides, etc., 29.5% Gum along with other carbohydrates, Mineral matter 11%, cellulose as well as the other components 6.7% by difference. Yeast has a great contribution to the survival and growth of honey bees especially at the larval stage and increased the larvae survival up to 80% and 30% [30]. Vandenberg and Shimanuki [31] described in their study that the larval and adult body weight of honey bees can also be increased by the application of yeast, and yeast-mixed diet.

For optimum growth and development of bees, a defined quality of proteins is required. If nurse bees are not capable of acquiring pollen or other proper protein source, their brood food gland secretions are not sufficient for the normal growth and development of larvae and also egg production of the queen. So, as the yeast is composed of 51.8% protein, it strengthens the colony and increases the longevity of larvae. The results showed that *A. mellifera* larvae could be helpful in developing a mixture of supplements, yeast extract, and water without added carbohydrates. However, they cannot pupate and become adults if their diet does not contain enough carbohydrates [32].

5. CONCLUSIONS

It is concluded that the yeast is important for the bees' development. The Langstroth single deep, equalized, and uncontaminated colonies with comparable food resources should be located in a shady place preferably at good floral accessibility for the bees for natural foraging. The colonies should be placed possibly very far from the chemical-treated/sprayed fields. Brewers' yeast, non-floral protein diet for honey bees, sugar mixed diet should be applied in the colonies with proper

mentioned concentration. For better eggs and larvae development, the diet should be placed in the colony beside the frames in polythene bags of proper size and changed every other day with fresh ones. It should be in view that the colony is free of diseases and pests and extra cells in the colonies must be removed.

6. RECOMMENDATIONS

Based on the findings of the current study, it is recommended that the yeast enhance the development of honey bee brood and may be used as a supplemental diet particularly in the dearth period when there is no pollen available in the fields.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

8. REFERENCES

1. J.B. Free (Ed.). Insect Pollination of Crops, (2nd edition). *Academic Press, London, UK* (1993).
2. M. Winston, W. Chalmers, and P. Lee. Effects of two pollen substitutes on brood mortality and length of adult life in the honeybee. *Journal of Apicultural Research* 22(1): 49-52 (1983).
3. R.C. Mishra, J. Kumar, and J.K. Gupta. A new approach to the control of predatory wasps (*Vespa* spp.) of the honeybee (*Apis Mellifera* L.). *Journal of Apicultural Research* 28(3): 126-130 (1989).
4. T.S.K. Johanson. Royal jelly. *Bee World* 36(2): 21-32 (1955).
5. G.A. Wright, S.W. Nicolson, and S. Shafir. Nutritional physiology and ecology of honey bees. *Annual Review of Entomology* (63): 327–344 (2018).
6. M.H. Haydak. Honey bee nutrition. *Annual Review of Entomology* 15(1): 143-156 (1970).
7. J. Herbert, W. Elton, and H. Shimanuki. Brood-rearing capability of caged honeybees fed synthetic diets. *Journal of Apicultural Research* 16(3):150-153 (1977).
8. J.O. Schmidt, C.T. Steven, and D.L. Marshall. Survival of honey bees, Apismellifera (Hymenoptera: Apidae), fed various pollen sources. *Annals of the Entomological Society of America* 80(2): 176-183 (1987).
9. H.R. Mattila, and G. W. Otis. Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies. *Journal of Economic Entomology* 99: 604–613 (2006).
10. T. Pankiw, R.R. Sagili, and B.N. Metz. Brood pheromone effects on colony protein supplement consumption and growth in the honey bee (Hymenoptera: Apidae) in a subtropical winter climate. *Journal of Economic Entomology* 101: 1749–1755 (2008).
11. R. Nabors. The effects of spring feeding pollen substitute to colonies of *Apismellifera*. *American Bee Journal* 140: 322-323 (2000).
12. N. Hrassnigg, and K. Crailsheim. Differences in drone and worker physiology in honeybees (*Apis mellifera*). *Apidologie* 36(2): 255-277 (2005).
13. D. Babendreier, N. Kalberer, J. Romeis, P. Fluri, and F. Bigler. Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants. *Apidologie* 35(3): 293-300 (2004).
14. B. Moritz, and K. Crailsheim. Physiology of protein digestion in the midgut of the honeybee (*Apis* mellifera L.). *Journal of Insect Physiology* 33(12): 923-931 (1987).
15. D.E. Shaw. The incidental collection of fungal spores by bees and the collection of spores in lieu of pollen. *Bee World* 71: 158-176 (1990).
16. E. Brouwers, R. Ebert, and J. Beetsma. Behavioural and physiological aspects of nurse bees in relation to the composition of larval food during caste differentiation in the honeybee. *Journal of Apicultural Research* 26(1): 11-23 (1987).
17. P. Aupinel, D. Fortini, H. Dufour, J. Tasei, B. Michaud, J. Odoux, and M. Pham-Delegue. Improvement of artificial feeding in a standard in vitro method for rearing Apis mellifera larvae. *Bulletin of Insectology* 58(2): 107-111 (2005).
18. M. Lindauer. Ein beitrag zur frage der arbeitsteilung im bienenstaat. *Zeitschrift* für *Vergleichende Physiologie* 34(4): 299-345 (1952).
19. T.K. Agrawal. Beekeeping industry in India: Future potential. *International Journal of Research and Applied Natural Science* 2(7): 133–140 (2014).
20. A. Amro, M. Omar, and A. Al-Ghamdi. Influence of different proteinaceous diets on consumption, brood rearing, and honey bee quality parameters under isolation conditions. *Turkish Journal of Veterinary and Animal Science* 40(4): 468–475 (2016).
21. G. DeGrandi-Hoffman, G. Wardell, F. Ahumada-Segura, T. Rinderer, R. Danka, and J. Pettis. Comparisons of pollen substitute diets for honey bees: consumption rates by colonies and effects on brood and adult populations. *Journal of Apiculture Research* 47(4): 265–270 (2008).
22. T.K. Gemeda. Testing the effect of dearth period supplementary feeding of honeybee (*Apis mellifera*) on brood development and honey production. *International Journal of Advance Research* 2: 319-324 (2014).
23. B. Madras-Majewska, Z. Jasinski, A. Jojczyk, and F. Korfanty. Effect of early supplemental feedinghoney bee colonies with a substitute of bee bread made of drone brood candy, glucose and honey on colony strength. *Journal of Apiculture Science* 49(1): 41–46 (2014).
24. A.M. Safari P.G. Kevan, and J.L. Atkinson. Feed-Bee: A new bee feed is added to the menu. *Bee Cult*ure 134(1): 47–48 (2006).
25. N. Wijayati, D.S. Hardjono, M. Rahmavati, and A. Kurniawati. Formulation of winged bean seeds as

pollen substitute for outgrowth of honeybees (*Apis mellifera* L.). *Journal of Physical Conference Series* 1321(2): 022040 (2019).
26. K.E. Anderson, T.H. Sheehan, B.J. Eckholm, B.M. Mott, and G. DeGrandi-Hoffman. An emerging paradigm of colony health: Microbial balance of the honey bee and hive (*Apis mellifera*). *Insectes Sociaux* 58(4): 431-444 (2011).
27. J.-H. Yun, M.-J. Jung, P.S. Kim, and J.-W. Bae. Social status shapes the bacterial and fungal gut communities of the honey bee. *Scientific Reports* 8(1): 2019 (2018).
28. J. Van der Steen. Effect of a home-made pollen substitute on honey bee colony development. *Journal of Apicultural Research* 46(2):114-119 (2007).
29. D. Somerville. Honey bee nutrition and supplementary feeding. *Agnote DAI/178. NSW Agriculture* pp. 1-8 (2000).
30. H. Rembold, and B. Lackner. Rearing of honeybee larvae in vitro: Effect of yeast extract on queen differentiation. *Journal of Apicultural Research* 20(3): 165-171 (1981).
31. J. Vandenberg, and H. Shimanuki. Technique for rearing worker honeybees in the laboratory. *Journal of Apicultural Research* 26(2): 90-97 (1987).
32. O. Kaftanoglu, T.A. Linksvaye, and R.E. Page. Rearing honey bees, Apismellifera, in vitro 1: effects of sugar concentrations on survival and development. *Journal of Insect Science* 11(96): 1-10 (2011).