

Artificial Diet Alternatives or Supplements for Healthy Honey Beekeeping

Abstract

This comprehensive review illuminates the crucial role of proper nutrition in the growth and development of honeybee colonies, adult bees, and larvae, emphasizing both the challenges and advantages associated with artificial diets. Covering a spectrum of topics, the discussion explores the formulation and applications of sugar syrups, the pivotal role of pollen substitutes, and the critical significance of a well-balanced diet rich in proteins, particularly vital for larval development. A significant focus is placed on the importance of adequate hydration and the utilization of electrolyte solutions, playing pivotal roles in supporting honeybee thermoregulation, digestion, and overall colony maintenance. Moreover, the review delves into the potential impact of vitamin C supplementation, shedding light on its role in enhancing honeybee health and immunity, and its capacity to mitigate stressors, with implications for overall colony survival. The intricate interconnections between colony nutrition, adult nutrition, and larval nutrition are underscored, emphasizing the pivotal role of trophallactic contacts in information transfer within the colony. The detailed examination of carbohydrate and protein needs at different developmental stages provides nuanced insights, taking into consideration various sources, variations, and potential risks associated with their consumption, thereby contributing valuable knowledge for effective honeybee management practices. Shifting the focus to the specific challenges faced by beekeeping in India, particularly during dearth periods, the study investigates various artificial diets fed to colonies. Special attention is given to the development of pollen substitutes, a critical aspect for enhancing beekeeping practices in the region. Insights gleaned from past attempts at creating artificial diets and their impact on colony parameters contribute to ongoing efforts to address the unique challenges confronted by Indian beekeepers. The review extends its scope to stingless bees, exploring the complexities of developing artificial diets for these species and highlighting the preference for fermented pollen. The physicochemical characteristics of pollen collected by specific stingless bee species provide valuable insights, offering potential avenues for future artificial diet development. Supplementary feeding strategies, including overwintering feeding, stimulation of brood rearing, prevention of starvation, colony building, and comb construction, are explored. Various artificial feeding methods, such as sugar syrups, protein supplements, pollen substitutes, and hive supplements, are discussed in their roles supporting different aspects of honeybee nutrition. The review concludes with a discussion on the potential benefits of vitamin C supplementation in bee nutrition, contributing to a broader understanding of artificial diets and supplementary feeding strategies tailored for the conservation and sustainable management of honeybee populations, especially within the context of the unique challenges faced by Indian beekeeping.

Keywords: Artificial diet, Colony Health, Honeybee Nutrition, Supplemental feeding

INTRODUCTION

Honeybees are social insects that require proper nutrition for growth and development in order to create a variety of bee products for human benefit as beekeeping is a successful enterprise in India [1]. Good nutrition for honeybee growth and development has been reported by numerous scientists. Hendriksma[2] documented that the amino acids required for the growth and development of honeybees, and Rodney & Purdy[3] analysis of the general dietary requirements of honeybees. “The seasonality of food resources has forced bees to develop survival strategies to deal with periods of food scarcity” [4]. “For example, solitary bees spend most of their lives as immature forms before emerging as adults before or during the peak bloom periods of specific plants. Generally, they reproduce quickly and die after provisioning brood cells, laying their eggs, and closing the nests, with offspring emerging only in the next rewarding season” [5].

Understanding the intricate nutritional requirements of honeybee colonies, adult bees, and larvae, and the challenges and benefits associated with artificial diets, is crucial for conserving and sustainably managing honeybee populations in an ever-changing environment. The review delves into the formulation and applications of sugar syrups, including varying sucrose-water ratios and their role in sustaining colonies. The effectiveness of pollen substitutes in supporting colony growth and their potential impacts on bee health are evaluated. Honeybees' need for a balanced diet rich in proteins, particularly vital for larval development, is discussed. The role of protein supplements, such as soy-based or yeast-derived options, in enhancing the nutritional profile of bee diets is addressed, along with optimal timing for protein feeding.

Adequate hydration's significance for honeybees, crucial for thermoregulation, digestion, and colony maintenance, is explored. The use of electrolyte solutions to provide essential ions and nutrients to bees, particularly during hot weather, is also discussed. Finally, the review delves into the proposed role of vitamin C (ascorbic acid) supplementation in enhancing honeybee health and immunity. Research regarding vitamin C's potential to mitigate the adverse effects of stressors, such as pesticides and pathogens, and its implications for colony survival are discussed.

Nutritional requirements for bees at different stages

Nutritional requirements for bees at different stages can be of three kinds as follows: Colony nutrition, Adult Nutrition and Larval nutrition. “In a colony, the nutritional levels are closely connected through numerous adult-brood interactions and trophallactic contacts”. [60] Trophallaxis describes the social transfer of food from one adult individual to another adult, partly in a directed manner, and partly generating a common stomach that enables all bees to obtain knowledge of the nutritional status of the colony” [6,7]. “Both adults and larvae are highly dependent on the food stored by the colonies, and adult honeybees may adapt their brood care strategies or forage according to the need and supply of carbohydrates and proteins” [8].

1. Carbohydrate

Colony nutrition—“The natural carbohydrate source of honeybees is nectar or honeydew, collected by the foragers from plants and transported to the hive and finally stored in sealed cells as honey. This transformation from nectar to honey is gradational and starts during the return flight” [9]. “In the colony, the water content is further reduced to 16– 20%” [10]. “The health of honeybee colonies is not only defined by the absence of diseases but also by the presence of many well-nourished individuals capable of producing progeny and resisting stressors such as parasites, infections, insecticides, and periods of a dearth” [11].

“In the colony, the water content is reduced to 16– 20%, and enzymes (diastase, invertase, and glucose oxidase) are added, which account for the sugar composition of honey: on average 38% fructose, 31% glucose, and other disaccharides and trisaccharide” [12]. Annual honey yields vary widely, depending on climate, beekeeping operation, and forage availability, and are reported to be 19.2 kg [13], and 24.3–31.3 kg [14]. “Annual yields may reach 96–124 kg per colony in Canada” [15]. “Carbohydrates are fed to colonies daily after harvesting honey or during the dearth period. This is fulfilled by feeding sucrose solution, inverted sugars, HFCS (high fructose corn syrup), or various fruit syrups inside the hive” [16]. “Grape syrup causes dysentery and reduces longevity, and its use is therefore not recommended” [17]. “In hot regions there are only a few nectar sources available, feeding of HFCS that has been stored for a long time should be avoided” [18].

Adult Nutrition —“Carbohydrates meet the energetic expenses of honeybees. Adult workers are dependent on colony food stores and do not survive long periods without feeding as they

do not have carbohydrate, protein, or lipid reserves in their bodies” [19]. “An adult honeybee worker needs about 4 mg of utilizable sugars per day for survival” [20]. “Some sugars, like mannose, galactose, arabinose, xylose, melibiose, raffinose, stachyose, and lactose are toxic to bees. About 40% of the sugars found in soybeans, which are used as pollen substitutes, are toxic to bees. Toxicity is reduced when honeybees have plenty of nectar or when these carbohydrates are experimentally diluted to under 4% with 50% sucrose solution” [21]. “Another substance toxic to bees is hydroxymethylfurfural (HMF), formed from the acid-catalyzed dehydration of hexose sugars, especially fructose, and formed in honey because of heat treatment or storage. High HMF levels must also be considered as a risk in the feeding of inverted sugars or HFCS”[22].

Larvae Nutrition – “A larvae is fed by nurse bees and regularly inspected by them, so that it is always sufficiently provided with food. The sugar content (fructose and sucrose) in the brood food is 18% in the first three days of larval development, then 45% in the last two days” [23].

Carbohydrate needs would be higher when considering the costs of adult bees to provide the stable environment necessary for brood rearing (like comb building or thermoregulation). A lack of carbohydrates limits the number of larvae reared in spring when sources of nectar are poor and winter stores are almost finished, or after harvesting the honey without replacement of carbohydrates. Little is known about the lethal or sublethal effects of HMF (hydroxy methyl furfural) or toxic sugars on the development of larvae.

2. Protein

Colony nutrition – “Pollen is the only natural protein source for honeybees. Colonies collect 10–26 kg of pollen per year estimated the pollen requirement of two 10-frame colonies to be 13.4 kg per year and the pollen requirement of two 10-frame colonies to be 17.8 kg per year” [24]. “In the colony, honeybees mix pollen with regurgitated nectar, honey, and glandular secretions to produce bee bread, which differs from freshly collected pollen, in having a lower pH and less starch” [25]. “The protein content of pollen from different species and regions varies widely (2.5– 61%)”[26]. The protein content of bees also varies depending on the season.

Adult Nutrition–“Proteins make up 66–74% of the dry matter of adult workers” [27]. “The protein content of bees also varies depending on the season. In late summer, the last generation of bees produced shows distinct physiological characteristics” [28]. “The

difference is accompanied by the bees' capability of building up high levels of hemolymph proteins, which allow them to survive several months on carbohydrates only. Vitellogenin is the main storage protein in hemolymphs and precursor for many other proteins" [29]. "The importance of this lipoprotein for the onset of foraging by workers, longevity, and overwintering has been demonstrated" [30].

Larvae Nutrition—"Pollen is only fed directly to larvae in a small amount; most of the protein a larva obtains is processed brood food from adult honeybees calculated the portion of protein directly derived from pollen to be about 5%" [31]. "To rear one larva, 25–37.5 mg protein (or 125–187.5 mg pollen) is needed" [32]. "The number of feedings and the food applied by nurses is adjusted to the age of the larva. Young larvae are visited and fed less often than older larvae" [33].

"To precisely determine the effect of larval nutrition on adult honeybees one can use feeding protocols to rear larvae in vitro" [34]. "Honeybees reared according to these protocols have reduced body size and weight, especially dry weight of the thorax, compared to sister bees reared in a colony. Their flight capacity (measured in tethered flight in a roundabout) was like the controls, with minor drawbacks in high performance" [35]. "Other effects of larval malnutrition on lifetime flight performance or onset of foraging cannot be excluded. Ovaries of workers reared under low-pollen conditions are less developed compared with those of workers reared by colonies given access to a high-pollen diet". [60] [36] investigated "the effect of larval nutrition on the ovaries of queenright workers and demonstrated a carry-over of nutrients from the larval to the adult stage. When both groups were fed the same mixture of royal jelly and honey as adults, the ovaries of the bees reared under poor conditions did not develop as well as those of the others".

ARTIFICIAL DIET OF HONEY BEES

"In India, the major problem in beekeeping is maintaining the good strength of honeybee colonies during the period of dearth. In tropical and subtropical parts of India, summers are very harsh for honeybees as enough bee flora is not available during this period. The condition is more severe in central parts of our country where dearth periods are a little longer as compared to other parts. The dearth periods may result in dwindling and even death of bee colonies. Therefore, during these periods, special care should be taken for the bee's management. The first option is the migration of bee colonies, but it also includes lots of

time, money, and labor. Another option is to feed the bee colonies with protein protein-rich artificial diet so that brood-rearing activity can be enhanced and maintain the strength of colonies. The necessity of artificial diets for honeybees has been long long-standing interest to the beekeeping industries” [37,38].

“Different types of artificial diets have been formed and their effect on various colony parameters was observed by many researchers” [39]. “In the present study, an attempt has been made to compare the effect of various diet formulations fed to bee colonies during the period of dearth on colony parameters like bee strength, brood rearing, and the number of frames covered by bees, so that suitable pollen substitute can be developed to improve beekeeping practice in India. The concept of any animal domestication program is finding a substitute for a natural food source or the means to supplement diets. In the case of stingless bees, the beekeeper can easily replace the honey with artificial sugar solutions” [40]. “On the other hand, replacing pollen has not been an easy task because of the complexity of its fermentation and nutritional composition. An adequate artificial diet would drastically change the scenario of meliponid culture and improve the colony multiplication process, avoiding problems associated with the seasonality of floral resources. Efforts applied to develop pollen substitutes and some artificial diets are encouraging. Because fermentation is necessary for developing these diets, recent insights about the microbiota involved in pollen fermentation certainly help to improve artificial diet development”. [40]

“A pioneer defined an artificial diet for stingless bees. Fresh pollen, mixed with honey and fermented pollen, was fermented for about 2 weeks, and given to bees. The unfermented pollen was rejected by the workers” [41]. “A recent study confirms again that bees prefer fermented pollen, choosing bee bread instead of fresh material” [42]. Applying fermented pollen as a natural inoculum for the unfermented pollen seems to be the key to bee acceptance. By using fermented material, it was theorized that the established microbiota would be transferred to the unfermented pollen, and it would become more attractive and greater in nutritional value to the bees.

“Protein source substitutes have been tested and most recipes are simple. Generally, a mixture of different protein sources and honey or sugar is used, supplemented with naturally fermented pollen from the species that will receive the artificial diet. Before using the mixture, it is desirable to ferment it for several days” [43,44]. “Nevertheless, such pollen substitutes are frequently rejected by bees. The risk of causing problems to bees is not significant even if the diet is not accepted. However, beekeepers should carefully check the

hived colonies while providing artificial diets, because in case of rejection, the nutrient-rich material can accumulate and may also attract enemies to the colonies, such as phorid flies.

The physicochemical characteristics of pollen collected by the stingless bees *Melipona seminigra* and *M. interrupta* have huge potential to contribute to the development of artificial diets for these insects in the future. The results reveal that the composition of collected pollen by each species is 53.39 and 37.12% moisture, 37.63 and 24.00% protein, 10.81 and 6.47% lipid, 4.03 and 2.74% ash, 9.30 and 13.65% crude fiber, 25.66 and 44.27% carbohydrates, 350.47 and 331.33 kcal energy, a pH of 3.70 and 3.34, total solid content of 46.60 and 62.87%, and water activity of 0.91 and 0.85, respectively” [45].

Table 1. Reason for Supplementary Feeding

Reason for Supplementary Feeding	Brief Description Reference	Description
Overwintering		Honeybees need food reserves for surviving the winter when natural forage is scarce. Supplementary feeding ensures sufficient stores for cold months [46]
Stimulating Brood Rearing		Supplementary feeding during the brood-rearing season provides essential nutrition, stimulating the queen to lay more eggs and enhancing bee population growth [21]
Preventing Starvation		In times of dearth, supplementary feeding prevents colony starvation due to limited natural nectar and pollen sources, avoiding population decline or colony collapse [47]
Colony Building		Supplementary feeding aids in establishing new colonies or dividing existing ones by providing initial nutrition for rapid colony growth [48].
Comb Construction		Bees need energy to produce wax for comb building. Supplementary feeding provides carbohydrates crucial for wax production, honey storage, and brood rearing [49].

Types of Artificial Feeds

Sugar Syrups

Sugar syrups, primarily composed of sucrose and water, are among the most used artificial feeds for honeybees. Different syrup ratios, such as 1:1 (for stimulating brood production) and 2:1 (for winter feeding), are employed based on colony requirements.

Protein Supplements

Protein supplements are used to meet the nutritional needs of honeybees. Various sources, including soybean, pea, and pollen substitutes, are available to provide essential amino acids and proteins.

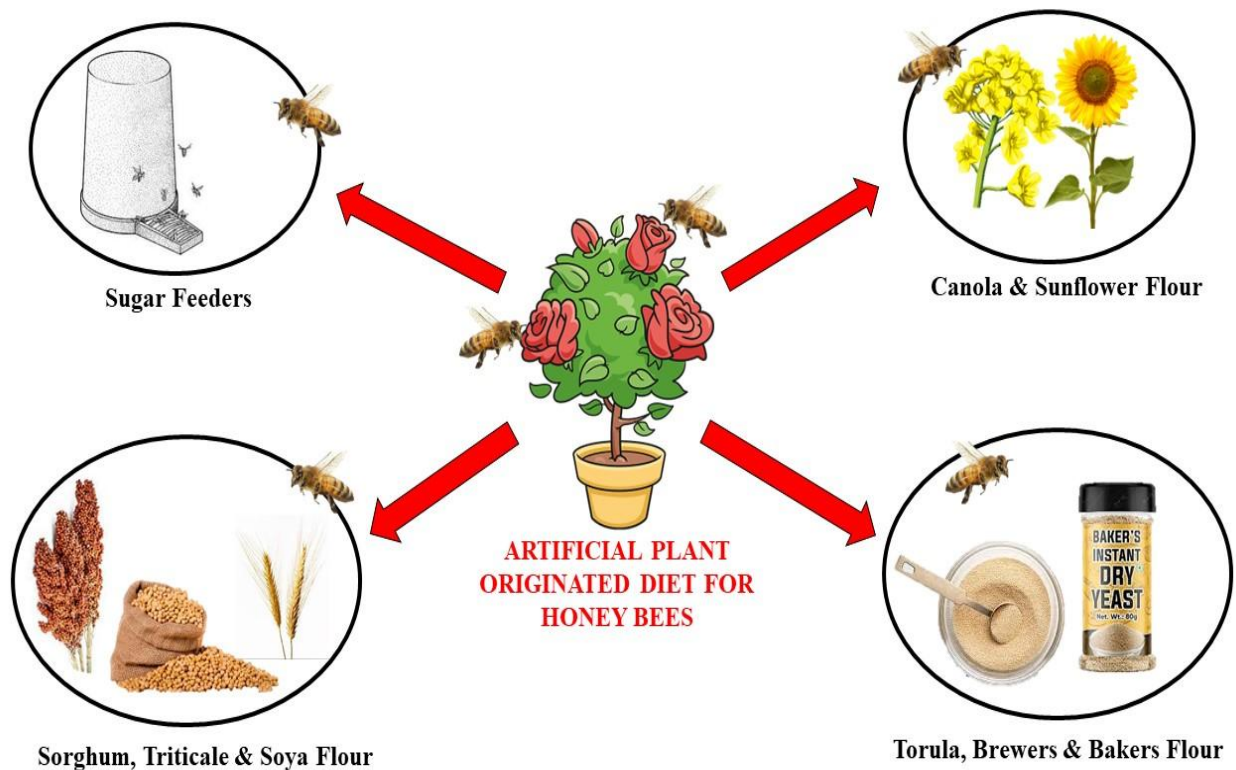
Pollen Substitutes

Pollen substitutes aim to replicate the nutritional value of natural pollen, which is crucial for the development of brood. They often include ingredients like soy flour, brewer's yeast, and vitamins to approximate the nutrient profile of pollen.

Hive Supplements

These include various supplements like vitamins, minerals, and essential oils, which are added to artificial feeds to boost honeybee health and immunity.

Fig 1. Various supplementary feed options for honeybees:-



1. Sugar Syrups:

Sugar syrups are a commonly used supplementary feed for honeybees. They provide carbohydrates, which are essential for energy. Different ratios of sugar and water are used for various purposes:**1:1 Ratio (50% Sugar):** This syrup, consisting of equal parts sugar and water, is often used in the spring and early summer. It provides bees with a quick source of energy, stimulating brood production and colony expansion [50].**2:1 Ratio (67% Sugar):** This syrup, with two parts sugar to one part water, is commonly used in the late summer and fall to help bees build up stores for the winter. It has a higher sugar concentration for better long-term storage [50].

2. Pollen Substitutes or Supplements:

Pollen substitutes or supplements are used when natural pollen is scarce or of poor quality. They aim to provide essential proteins and amino acids to the bees. These substitutes typically contain a mixture of plant-based ingredients, such as soy flour, brewer's yeast, and various nutrients. Pollen substitutes can include soybean flour, brewer's yeast, and other protein sources along with essential vitamins and minerals [51]. Pollen substitutes are especially useful during periods of pollen dearth, stimulating brood production, and maintaining colony strength [52]

3. Protein-rich Feeds:

Protein-rich feeds are designed to supplement the protein intake of bees. These feeds are vital for brood rearing and overall colony growth. Soybean flour and brewer's yeast are common protein sources for the diet of insects [53, 54]. Brewer's yeast is known for its high protein content, and it is often mixed with other ingredients [55]. Protein-rich feeds support the development of healthy brood and contribute to the overall health of the colony.

4. Fondant or Candy Boards:

Fondant and candy boards are solid, sugar-based feeds that provide a slow-release source of energy for bees. They are typically used during the winter months or when it's essential to provide a more extended food source. Fondant and candy boards are made by boiling sugar and water together, allowing it to harden, and then placing it inside the hive

[56]. Fondant and candy boards are suitable for winter feeding as they don't crystallize in low temperatures, providing bees with a consistent food source.

5. Water and Electrolyte Solutions:

Water is essential for bees, especially during hot weather. Electrolyte solutions can be added to water to help bees stay hydrated and replenish electrolytes lost during foraging. Proper hydration is critical for thermoregulation, wax production, and food processing within the hive. Electrolyte solutions are essential during heatwaves and periods of drought [57].

6. Vit C Supplementation in Bees Nutrition

Some research has demonstrated that when the colonies received vitamin C as supplement, they experienced a substantial reduction of approximately 33% in bee losses over the winter season. This underscores the potential advantages associated with the incorporation of vitamin C into the bee diet, particularly in terms of promoting overwintering survival rates [58, 59] which encompassed twelve distinct developmental stages ranging from the emergence of newly hatched larvae to the maturation of adult worker bees, yielded noteworthy insights into the fluctuating antioxidant profile during the bees' growth process. It was observed that newly emerged worker bees were particularly vulnerable to oxidative stress.

During winter and early spring four colonies were fed syrup containing 1.8 mg vitamin C per kg. Emerging worker bees exhibited notably elevated concentrations of both protein and glutathione. These compounds play crucial roles in antioxidative defense mechanisms [58]. Essential antioxidant enzymes, including catalase, peroxidase, and glutathione transferase, displayed heightened activities in workers who emerged from colonies receiving vitamin C supplementation. These enzymes are pivotal in combating oxidative stress and maintaining cellular health [59]. Importantly, the introduction of vitamin C did not result in any significant changes in the increase in brood weight. This suggests that while the antioxidant status was improved, overall growth and development of the brood remained unaffected. Worker bees that emerged from colonies with vitamin C supplementation exhibited higher protein levels compared to those in the control group. This indicates that their nutritional status may have been positively influenced by vitamin C [58].

Conclusion

This comprehensive review has illuminated the intricate world of artificial diets and supplements in honeybee nutrition, shedding light on their profound implications for honeybee health and sustainability. Honeybees, as pivotal pollinators in global agriculture, rely on diverse sources of nutrition, each contributing to their overall well-being, productivity, and resilience. In summary, this review has highlighted several key takeaways, the collective diet of a honeybee colony, encompassing honey and pollen stores, plays a vital role in ensuring colony survival during times of scarcity. Understanding the interplay of these resources and their responses to environmental stressors is crucial for effective hive management. The nutritional needs of worker bees, drones, and queens are multifaceted, varying with age, caste, and season. Supplemental feeding, including sugar syrups, has been a crucial tool in sustaining adult bee health, particularly in resource-deficient periods. The nutritional quality of larval diets, whether natural or artificial, profoundly impacts the development and ultimate health of adult bees. An understanding of the composition of royal jelly, bee bread, and other larval foods is essential for beekeepers aiming to foster strong, healthy colonies. In the face of these challenges, it is clear that honeybee nutrition is a dynamic and evolving field. Research into artificial diets and supplements continues to refine our understanding of their benefits and limitations. Effective hive management should remain an adaptive process, taking into account the specific needs of each colony and the ever-changing environmental conditions. Ultimately, the sustainability of honeybee populations and their critical role in global agriculture hinge on our ability to provide them with the nutrition required to thrive. Beekeepers, researchers, and policymakers must work together to further advance our knowledge of honeybee nutrition, enabling us to safeguard these invaluable pollinators for the benefit of both ecosystems and human society.

REFERENCES:

1. Narang A, Kumar D, Gupta G. Political, economical, social, technological and SWOT analysis of beekeeping as a successful enterprise in India: An overview. *Journal of Applied and Natural Science* 2022; 14 (1), 194-202
2. Hendriksma HP, Pachow CD, Nieh JC. Effects of essential amino acid supplementation to promote honey bee gland and muscle development in cages and colonies. *Journal of insect physiology*. 2019;117:103906.

3. Rodney S, Purdy J. Dietary requirements of individual nectar foragers, and colony-level pollen and nectar consumption: a review to support pesticide exposure assessment for honey bees. *Apidologie*. 2020;51(2):163-79.
4. Li C. Understanding, Conservation, and Protection of Precious Natural Resources: Bees. *Environmental and Natural Resources Engineering*. 2021;1-51.
5. Michener CD. The meliponini. In *Pot-honey: a legacy of stingless bees 2012* ; (pp. 3-17).
6. Crailsheim K. Interadult feeding of jelly in honeybee (*Apis mellifera* L.) colonies. *Journal of Comparative Physiology B*. 1991;161:55-60.
7. Crailsheim K. Trophallactic interactions in the adult honeybee (*Apis mellifera* L.). *Apidologie*. 1998;29(1-2):97-112.
8. Schmickl T, Crailsheim K. Inner nest homeostasis in a changing environment with special emphasis on honey bee brood nursing and pollen supply. *Apidologie*. 2004;35(3):249-63.
9. Nicolson SW, Human H. Bees get a head start on honey production. *Biology letters*. 2008 ;4(3):299-301.
10. Mitchell D. Nectar, humidity, honey bees (*Apis mellifera*) and *Varroa* in summer: a theoretical thermofluid analysis of the fate of water vapour from honey ripening and its implications on the control of *Varroa* destructor. *Journal of the Royal Society Interface*. 2019;16(156):20190048.
11. Brodschneider R, Crailsheim K. Nutrition and health in honey bees. *Apidologie*. 2010;41(3):278-94.
12. Crăciun ME, Parvulescu OC, Donise AC, Dobre T, Stanciu DR. Characterization and classification of Romanian acacia honey based on its physicochemical parameters and chemometrics. *Scientific Reports*. 2020 ;10(1):20690.
13. Reda GK, Girmay S, Gebremichael B. Beekeeping practice and honey production potential in Afar Regional State, Ethiopia. *Acta Universitatis Sapientiae, Agriculture and Environment*. 2018;10(1):66-82.
14. Avni D, Dag A, Shafir S. The effect of surface area of pollen patties fed to honey bee (*Apis mellifera*) colonies on their consumption, brood production and honey yields. *Journal of apicultural research*. 2009;48(1):23-8.
15. Mattila HR, Otis GW. Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies. *Journal of economic entomology*. 2006;99(3):604-13.

16. Neupane KR, Thapa RB. Alternative to off-season sugar supplement feeding of honeybees. *Journal of the Institute of Agriculture and Animal Science*. 2005;26:77-81.
17. Barker RJ, Lehner Y. Laboratory comparison of high fructose corn syrup, grape syrup, honey, and sucrose syrup as maintenance food for caged honey bees. *Apidologie*. 1978;9(2):111-6.
18. LeBlanc BW, Eggleston G, Sammataro D, Cornett C, Dufault R, Deeby T, St. Cyr E. Formation of hydroxymethylfurfural in domestic high-fructose corn syrup and its toxicity to the honey bee (*Apis mellifera*). *Journal of Agricultural and Food Chemistry*. 2009;57(16):7369-76.
19. Hrassnigg N, Crailsheim K. Differences in drone and worker physiology in honeybees (*Apis mellifera*). *Apidologie*. 2005;36(2):255-77.
20. Kim HJ, Seo GB, Ullah Z, Kwon HW. Nutrition for Honey Bee to Prevent Colony Collapse. *Journal of Apiculture*. 2022;37(4):397-404.
21. Brodschneider R, Crailsheim K. Nutrition and health in honey bees. *Apidologie*. 2010;41(3):278-94.
22. Jachimowicz T, El Sherbiny G. Zur problematik der verwendung von invertzucker für die bienenfütterung. *Apidologie*. 1975;6(2):121-43.
23. Moliné MD, Fernández NJ, Damiani N, Churio MS, Gende LB. The effect of diet on *Apis mellifera* larval susceptibility to *Paenibacillus* larvae. *Journal of Apicultural Research*. 2020;59(5):817-24.
24. Agarwal R, Bansal A, Saini AS, Raj A, Kumar A, Gharde SK. Bee nutrition and artificial food.
25. Ellis AM And Hayes Jr GW. An evaluation of fresh versus fermented diets for honey bees (*Apis mellifera*). *Journal of apicultural research*. 2009;48(3):215-6.
26. Roulston TA, Cane JH, Buchmann SL. What governs protein content of pollen: pollinator preferences, pollen–pistil interactions, or phylogeny?. *Ecological monographs*. 2000;70(4):617-43.
27. Hrassnigg N And Crailsheim K. Differences in drone and worker physiology in honeybees (*Apis mellifera*). *Apidologie*. 2005;36(2):255-77.
28. Carroll MJ, Brown NJ, Ruetz Z, Ricigliano VA, Anderson KE. Honey bee retinue workers respond similarly to queens despite seasonal differences in Queen Mandibular Pheromone (QMP) signaling. *Plos one*. 2023;18(9):e0291710.

29. Isani G, Bellei E, Rudelli C, Cabbri R, Ferlizza E, Andreani G. SDS-PAGE-Based Quantitative Assay of Hemolymph Proteins in Honeybees: Progress and Prospects for Field Application. *International Journal of Molecular Sciences*. 2023;24(12):10216.
30. Amdam GV, Hartfelder K, Norberg K, Hagen A, Omholt SW. Altered physiology in worker honey bees (Hymenoptera: Apidae) infested with the mite *Varroa destructor* (Acari: Varroidae): a factor in colony loss during overwintering?. *Journal of economic entomology*. 2004 Jun 1;97(3):741-7.
31. Babendreier D, Kalberer N, Romeis J, Fluri P, Bigler F. Pollen consumption in honey bee larvae: a step forward in the risk assessment of transgenic plants. *Apidologie*. 2004;35(3):293-300.
32. Hrassnigg N, Crailsheim K. Differences in drone and worker physiology in honeybees (*Apis mellifera*). *Apidologie*. 2005;36(2):255-77.
33. Schmickl T, Crailsheim KJ. How honeybees (*Apis mellifera* L.) change their broodcare behaviour in response to non-foraging conditions and poor pollen conditions. *Behavioral Ecology and Sociobiology*. 2002;51:415-25.
34. Aupinel P, Fortini D, Dufour H, Tasei J, Michaud B, Odoux J, Pham-Delegue M. Improvement of artificial feeding in a standard in vitro method for rearing *Apis mellifera* larvae. *Bulletin of insectology*. 2005;58(2):107.
35. Brodschneider R, Haidmayer C, Riessberger-Gallé U, Crailsheim K. Protein uptake in honeybee colonies supplemented with two protein diets simultaneously. *Apidologie*. 2009;40:662.
36. Hoover SE, Higo HA, Winston ML. Worker honey bee ovary development: seasonal variation and the influence of larval and adult nutrition. *Journal of Comparative Physiology B*. 2006;176:55-63.
37. Buttstedt A, Pirk CW, Yusuf AA. Mandibular glands secrete 24-methylenecholesterol into honey bee (*Apis mellifera*) food jelly. *Insect Biochemistry and Molecular Biology*. 2023;161:104011.
38. Kumar M, Abrol DP, Sharma D, Vikram US, Singh AK. Impact of artificial diets on performance of *Apis mellifera* colonies during dearth periods. *J. Entomol Zool. Stud*. 2021;9(3):404-9.
39. Sihag RC, Gupta M. Development of an artificial pollen substitute/supplement diet to help tide the colonies of honeybee (*Apis mellifera* L.) over the dearth season. *Journal of Apicultural Science*. 2011;55(2).

40. Noiset P, Cabirol N, Rojas-Oropeza M, Warrit N, Nkoba K, Vereecken NJ. Honey compositional convergence and the parallel domestication of social bees. *Scientific Reports*. 2022 ;12(1):18280..
41. Menezes C, Paludo CR, Pupo MT. A review of the artificial diets used as pot-pollen substitutes. *Pot-Pollen in Stingless Bee Melittology*. 2018:253-62.
42. Vollet-Neto A, Maia-Silva C, Menezes C, Imperatriz-Fonseca VL. Newly emerged workers of the stingless bee *Scaptotrigona aff. depilis* prefer stored pollen to fresh pollen. *Apidologie*. 2017;48:204-10..
43. Costa L, Venturieri GC. Diet impacts on *Melipona flavolineata* workers (Apidae, Meliponini). *Journal of apicultural research*. 2009;48(1):38-45.
44. RABELO N, Venturieri GC, Contrera FA. Elaboração de uma dieta artificial protéica para *Melipona fasciculata*. 2009.
45. Rebelo KS, Ferreira AG, Carvalho-Zilse GA. Physicochemical characteristics of pollen collected by Amazonian stingless bees. *Ciência Rural*. 2016;46:927-32.
46. Van Dooremalen C, Stam E, Gerritsen L, Cornelissen B, Van der Steen J, Van Langevelde F, Blacquièrre T. Interactive effect of reduced pollen availability and *Varroa* destructor infestation limits growth and protein content of young honey bees. *Journal of insect physiology*. 2013 ;59(4):487-93.
47. Johnson RM. Honey bee toxicology. *Annual review of entomology*. 2015;60:415-34.
48. Marting PR, Koger B, Smith ML. Manipulating nest architecture reveals three-dimensional building strategies and colony resilience in honeybees. *Proceedings of the Royal Society B*. 2023;290(1998):20222565..
49. Castaños CE, Boyce MC, Bates T, Millar AH, Flematti G, Lawler NG, Grassl J. Lipidomic features of honey bee and colony health during limited supplementary feeding. *Insect Molecular Biology*. 2023.
50. DeGrandi-Hoffman G, Chen Y. Nutrition, immunity and viral infections in honey bees. *Current opinion in insect science*. 2015;10:170-6..
51. Taha EK and Al-Kahtani SN. Different feeding sources used for mass rearing of honeybee queens in Saudi Arabia. *The Journal of Animal and Plant Sciences*. 2005;15(2), 81-84.
52. Al-Kahtani SN and Taha EK. Comparative studies on the influence of pollen substitutes on the performance of *Apis mellifera* queens in cages. *Journal of King Saud University-Agricultural Sciences*. 2006;18(2), 113-126.

53. Gupta G, Yadav SR, Bhattacharya AK. Influence of synthetic plant growth substances on the survivorship and developmental parameters of *Spilarctia obliqua* Walker (Lepidoptera: Arctiidae). *Journal of Pest Science*. 2009;82:41-6.
54. Gupta G, Kumar NR. Growth and development of ladybird beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), on plant and animal based protein diets. *Journal of Asia-Pacific Entomology*. 2017 ;20(3):959-63.
55. Hoover SE, Higo HA, Winston ML. Worker honey bee ovary development: Seasonal variation and the influence of larval and adult nutrition. *Journal of Comparative Physiology B*. 2006 ;176:55-63..
56. Webster TC and Delaplane KS. Overwintering honey bee colonies: laboratory studies on the effects of sugar feeding on colony survival and honey production. *Journal of Apicultural Research*. 2001;40(1-2), 37-45.
57. Fewell JH, Bertram SM. Division of labor in a dynamic environment: response by honeybees (*Apis mellifera*) to graded changes in colony pollen stores. *Behavioral ecology and sociobiology*. 1999;46:171-9.
58. Farjan M, Łopieńska-Biernat E, Lipiński Z, Dmitryjuk M, Żóltowska K. Supplementing with vitamin C the diet of honeybees (*Apis mellifera carnica*) parasitized with *Varroa destructor*: effects on antioxidative status. *Parasitology*. 2014;141(6):770-6.
59. Tawfik AI, Ahmed ZH, Abdel-Rahman MF, Moustafa AM. Influence of winter feeding on colony development and the antioxidant system of the honey bee, *Apis mellifera*. *Journal of Apicultural Research*. 2020;59(5):752-63.
60. Brodschneider R, Crailsheim K. Nutrition and health in honey bees. *Apidologie*. 2010;41(3).