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Artificial Diet Supplementation: A Review for Sustainable Approach to Boost Honeybee Health

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Honeybees play an important role in ecosystem management and agriculture productivity with their pollination services. They are helpful in production of 35% of global food, which requires animal pollination. However, they face many challenges for their survival and development. These includes some of reasons like habitat loss, climate change, the use of insecticides in modern agriculture practices, loss of natural flora, and change in temperature. A declining honeybee population poses a threat to both ecological balance and food security. In response artificial diets are the emerging source to rescue the bees to supplement natural forage in unfavorable conditions to overcome bees from the nutrient deficiency and potential threats. However, the use of artificial diet on honeybee health remains poorly understood within a broader ecosystem context. This paper aims to provide the information of current understanding of effects of artificial diets on honeybee health, considering ecological conditions. We evaluate the nutritional composition of natural diet and compared it with the nutritional composition of artificial diets, their effect on honeybee's colony health, physiology, colony dynamics, immune functions, and their ecological outcomes at the population and community levels. The artificial diets have the positive effects on the bee health in the absence of natural food and dearth period. Additionally we explore how artificial diet influence honeybee behavior such as foraging and examine the gene expression and diseases resistant quality of the diets. The studies show that these diets may relieve immediate nutritional deficiencies; they also interrupt the natural forage behavior and interactions with native flora, particularly leading to the unplanned ecological conditions. We call for future research to close the knowledge gaps and modify the use of artificial diets while maintaining the integrity of the ecosystem.

Key words: Artificial diet, Bee nutrition, Colony development, Dearth period, Honey bees, Natural diet

Introduction

Honeybees hold significance in both ecological and social perspective. They play the role of great priority in sustaining ecosystems and beneficial in human health and well-being. They are the vital pollinators that helps in the reproduction of many different plant species, promoting both ecosystem and worldwide agriculture.[1] It is the most common single species pollinator globally which is known for the production of 35% global food, relying on animal pollination for reproduction, promoting economic, food security and sustainable agriculture.[2] They provide highly valuable pollination service to crops, during the time of food collection they carefully visit the flowers to gather nectar and pollen without harming the blooms or plants in this process.[3] By pollinating the different type of plant species, they support to the constancy and dependability of ecosystem. They promote the development of vegetation that serves as the food source and habitat for different organism, including from insects to mammals. Honeybees are essential to the performance to ecosystems, influencing nutrient cycling, soil fertility and overall ecosystem health.

Bees produce various products such as honey, propolis, royal jelly, bee venom, bee pollen, bee brood, and bee's wax. Honey is an energy-rich food source and has been used in traditional medicine for centuries. At least 200 compounds have been identified in honey such as; organic acids, minerals, proteins, amino acids, carbohydrates, water, flavonoids, phenolic acids, and other phytochemicals which are employed as medicines and nutritional supplements. It is significant for its antibacterial and antioxidant effects due to its enzymatic and non-enzymatic characteristics[4] and as an alternative treatment for women's medical gynecological problems for example hayit honey produced by the hayit plant (Vitex agnus-castus L.) has been used as a natural cure for gynecological issues in women and has been discovered to have aphrodisiac properties. Certain gynecological issues can be brought back to normal by the plant's dopaminergic qualities.[5] It also contains vitamins, minerals, hormones, and

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diterpenes. Worker bees gather propolis, a resinous substance, from plants and apply it to hive walls as a strong antibiotic. It has been used for wound care, oral disinfectants, and corpse embalming for a very long time. In many nations, propolis is being recognized as a possible health food that can help avoid conditions like microbial infections, diabetes, cancer, heart disease, and inflammation.[6] Royal jelly, produced by honeybees, has various medicinal, nutritional, cosmetic, and commercial uses. It is used as wound healer since ancient times, has anti-inflammatory, anticancer, anti-allergic, and antioxidant properties. Its constituents, Defensin-1 and 10-hydroxy-2-decenoic acid (10-HDA), improve wound healing by blocking p38 phosphorylation and NF-KB nuclear translocation. Royal jelly is a potent medication and dietary supplement due to its phenolic chemicals, lipids, fatty acids, wax, and steroids.[7] Bee venom and beeswax also impart therapeutic properties due to their bioactive compounds.[8] Bee pollen is rich in polyphenols a phenolic chemicals which is good for human health because they prevent biomolecular oxidation and decrease oxidative stress. It is a natural food supplement with antioxidant and radical scavenging activity[9] and also alternative of medicines. Bee bread also comprses flavonoid glycosides and shows therapeutic properties[10] Bee brood, primarily pupae, is a valuable human food with 18% protein, 119 I.U. of vitamin A, and 7000 I.U. of vitamin D per gram. It is found to be an excellent source of energy while Beeswax serves as a naturally occurring and potent skin barrier-preserving agent that relieves inflammation, lessens burns, and hydrates the skin. Additionally, it decreases the expansion of skin-associated microorganisms, minimizing illnesses linked to tinea infections.[11]

So honeybees are essential organisms as far as their products therapeutic utility is concerned. They are also responsible for pollinating wild plants and crops, securing the food production globally with ecosystem stability but now a day there are serious threat to the loss of bee colonies. There are multiple factors which are responsible for the loss of bee's colonies including loss of habitat, pesticide use, pathogens and change in climatic conditions. The phenomenon of colony loss is also as the colony collapse disorder (CCD). Also during the unfavorable conditions when there is scarcity of food and decrease in natural flora known as the dearth period of bees. To solve the problem researchers found a solution of artificial feeding or supplementary feeding to the bees during the unfavorable conditions or the dearth period to rescue the honeybee colonies. Researchers and beekeepers used various methods to provide artificial diets to bees by formulating the diet which mimics the composition of their natural nutrient requirement. This provides the positive result towards the stabilization of bee colony and has positive result on the honeybee health like on the size of glands, bee secretions, honey production, numbers of bees in the hive, bee diseases, and immune modulator and increase their gut microbiota. The artificial feeding is a good source to protect the bees' colonies from these unfavorable conditions.

Nutritional Requirement of Honeybees

Honeybees are important pollinators for agricultural. They live on stored honey and pollen throughout the winter period and cluster into a ball to conserve warmth. All honeybees are social and cooperative insects. They have some basic requirement of nutrition like other organisms for their basic body growth and development throughout the life in different stages. This requirement of nutrition is full fill by bees collecting nectar from the flowers and plants which is the natural diet of bee colony. Their nutritional requirement is different at colony level, larva and in adult stage of life.[12] The food that is stored in colony, the primary source of food for both larva and adults. Adult honeybees may modify their foraging as well as brood-care techniques in response to the availability and demand of proteins and carbohydrates. Insufficient pollen stores can have a destructive impact on quality of adult bees and ensures the rearing of offspring.[13] Drones, workers, queen bees have different requirements of nutrition according to their physiology.

Colony Nutritional Requirement

Nectar or honeydew is the natural source of carbohydrates for honeybees, which is progressively transformed into honey during their flight back.[14] Honey contains 38% fructose, 31% glucose, and additional disaccharides and trisaccharides as sugars. Yields of honey produced each year vary according on the environment, kind of beekeeping, and availability of feed.[15] In absence of food sources honeybees are able to survive because of their long-term provisioning and food storage. Honeybees rely on pollen for protein, collecting 10–26 kg per year.

They store a small amount of pollen and mix it with secretions, honey and nectar to formulate bee bread. This pollen has a lower amount of starch and less pH, providing higher nutritive value to honeybees.[16] The pollen's quality is attributed to microorganisms like *Lactobacillus and Bifidobacterium*.[17]

Adult Nutritional Requirement

Honeybees are mainly dependent on carbohydrates for their energy requirements. However adulthoneybee workers depend on food stored in the colony and are unable to sustain themselves for long period of time due to insufficient glycogen levels which is stored carbohydrate in the body. For their survival, they require roughly 4 mg of utilizable carbohydrates daily and sucrose is the most beneficial sugar for housed bees. Sugars like arabinose, stachyose, galactose, lactose, melibiose, raffinose, mannose, and xylose are poisonous to honeybees which are found in the pollen and nectar of plant like *Tiliaceae or Theaceae*.[12] Adult worker bees' dry weight is composed of 66–74% proteins, which rises during the initial few days as a result of amino acid anabolism.[18] Bees reach a maximum pollen consumption of 3.4–4.3 mg/day at the young age of nurses. Seasons affect the amount of protein required for adult bees, in the end of summer seasons bees exhibit unique physiological traits and a longer life span. When the winter season begins, vitelogenin which is the main protein stored in the haemolymph of the bees, necessary for honeybees older than twenty days to survive, find food, and hibernate.[19]

Larval Nutritional Requirement

Larvae are inspected and fed by nurse bees on a daily basis. The sugar content required by larvae varies from 18% in the initial three days to 45% in the final two days.[12] The carbohydrates requirement of a worker larva is 59.4 mg, particularly in the spring when there are fewer nectar sources and fewer honey harvests.[20] To raise one larva 125–187.5 mg of pollen or 25–37.5 mg of protein are required. Adult honeybee's process the majority of the protein which is needed by larvae, with plant pollen providing 5% of the total protein.[21] As the larvae get older, nurse bees modify feeding schedules and food application to provide them with high-quality food made from protein extracted from pollen. Young larvae are fed less frequently. Lipids, vitamins, and minerals are necessary for the existence and development of honeybees.[22] While extra nutrients can be provided to colonies by supplemental feeding. During the brood stage, workers metabolize lipids and can transfer specific sterols to larvae. Water-soluble vitamins can be found in pollen, however, there is no connection between vitamin C levels and the quantity of offspring produced.[22] For bees to survive, water is vital.

Honeybees and their Loss

Honeybees feed mostly on nectar and pollen that they collect from blooming plants. The main supply of carbohydrates for these insects is nectar, and pollen offers vital proteins, lipids, vitamins, and minerals for their general health.[23] Dearth periods are time of scarcity of nectar and pollen for bees, leading to reduced foraging activity, decreased brood production, and increased competition among bees. Bees rely on stored resources like honey and pollen, and may consume stored bee bread.[24] Competition can lead to conflicts and aggression, and if prolonged, bee colonies may experience weight loss and population decline. Behavioral adaptations may include exploring new food sources, foraging efficiency, or swarming. Nevertheless, a large variety of issues including pesticide use, bee's habitat loss and change in climate are progressively compromising the quantity and quality of these natural food sources.[25] Scientists dubbed this phenomena as colony collapse disorders (CCD) due to the severity and peculiar circumstances of these colony decreases.[26] According to reports, the majority of states' beekeepers has been impacted. With all factors considered, the numeral of controlled honeybee colonies fall by an average of 31.8% and 35.8% throughout the overwinter months of 2006/2007 and 2007/2008, respectively. For the winter of 2008–2009, the initial loss projections are 28.6%.[27] The specific causes of colony losses remain unknown at this time. Colony losses of honeybees are not unusual. The reasons for these losses are still mostly unknown, but the losses in recent years have been rapid and large-scale compared to previous times. Additionally, the primary cause of these losses is the bees' failure to return to the hive, which is largely inconsistent with their behavior.[28]

Based on the facts presented during the past three years addressing the many possible root causes of Colony Collapse Disorder, the USDA came to the conclusion in its 2007–2008 improvement report (discharged by June 2009) shows that currently there is no one contributor alone holds the responsibility for the condition.[29] This is the reason why researchers are

currently looking at the concept of CCD. It is a disorder caused by a number of distinct variables working in conjunction.[30] Insecticides that might be harming honeybees in different ways isn't anticipated. A novel parasite or pathogen, including viruses or the parasite *Nosema ceranae*, that could be attacking honeybees.[31] A confluence of current challenges could weaken bees' immune systems and disturb their social structures, increasing the likelihood of disease and colony collapse.[30] High rates of Varroa mite infection, low crop pollination nutritional value and apiary congestion, lack of nectar or pollen influence to constricted or infected water supplies, and migration-related stress are a few possible pressures.[27]

Composition of Artificial Diet

To overcome the problem, scientists are looking into artificial diets as a possible addition to or replacement for honeybees' natural diet. Bees eat artificial diets, which can complement or replace the natural nectar and pollen found in flowers. They are employed in beekeeping operations for commercial pollination or honey production, as well as in urban or drought-prone areas. Sugar, soybean, gram and milk powder are typical constituents of bees' artificial diet. The dietary requirements of bees as well as aspects like acceptability, digestibility, and shelf stability are taken into account during formulation. Artificial diets cannot fully replace real forage because the former offers extra advantages like enzymes and bio-active substances.[32] In order to preserve their health, life, and production, bees need a distinct and well-balanced diet. Three main components make up a bee's diet are nectar, pollen, and water. The main root of carbohydrates for honeybees is nectar, and it's extracted from flowers and processed to create honey. Honey is a food source for bees and also serves as a vital food source during the winter months when there is not enough nectar. The direct source of protein is pollen for bees[33] which is made of flowers and stored as "bee chuck" in the hive. For the construction and upkeep of their bodies, including their muscles, glands, and reproductive organs, bees require protein. In addition, water is a vital component of a bee's diet because it helps with food digestion, hive temperature regulation, and the adulteration of honey for human consumption. Apart from nectar, pollen and water, bees require a range of micro nutrients such as vitamins, minerals, and fatty acids.[34] Pollen, honey, and other plant materials are just a few of the many sources of these micro nutrients. To ensure that your bees have access to a healthy, well-balanced diet, it's crucial to provide them with a variety of floral sources. When nectar and pollen are scarce, additional feeding of sugar syrup and pollen backups may also be required. All things considered, bee production and health depend on a diversified and balanced diet and robust and healthy honeybee colonies depend on enough nourishment. Although artificial food can be a helpful tool for beekeepers to supplement their bees' diet during times of failure or to provide fresh nutrients for their colonies, bees mostly rely on natural sources of nectar and pollen for sustenance.[23]

Feeding

Feeding honeybees includes providing supplemental food substances like sugar syrup or pollen substitutes to support their nutritional requirements. This practice is particularly important during the times of low nectar availability or in the regions with limited forage availability. Proper feeding helps to maintain colony strength, supports brood rearing and improves overall bee health contributing to their survival and productivity.

Feeding Methods

Installation of a Sugar Feeder in Hive

Beekeepers employ various techniques to provide artificial food to their colonies, and one of them involves installing a sugar feeder within the hive.[35] The season and colony strength are the primary determinants of the amount of sugar supplied to the colony. Beekeepers mostly utilize sugar feeders as a form of container to replenish the bee colony's food supply on dry days when natural nectar and pollen are scarce in the open. When the weather is bad, the feeders are an excellent method to keep the colonies alive and reduce the amount of time they spend outside the hive to find food. The most common and dependable method used by beekeepers to support their colony's efficient and worry-free growth is feeding sugar to the bees. When utilizing these feeders, beekeepers just have to worry about the proportion of syrup given to each colony.[23]

Patties Formation

Patties made without pollen are known as pollen substitutes. These are offered in the market for sale and are made to resemble genuine pollen's nutritional composition.[36] Usually, a combination of proteins,

lipids, vitamins, and minerals are present in these patties. We can make them in homes and are known as homemade patties, using substances like soy flour, brewer's yeast, and other protein sources, beekeepers can also make their own patties that serve as pollen substitute.[37] A tiny bit of sugar syrup or honey can be added to the patties to increase their appeal to bees.[38]

Suction or Drip Feeding

Suction feeding or drip feeding is provided in a cage. Few beekeepers use drip systems, which continuously supply liquid feed.[39] First step is to fill the jar and other tools with a semi-liquid diet, place the jar in the cage with tiny numerous apertures, and let the food drip out of the holes so that the bees can collect it. This method is widely used in large-scale feeding initiatives.[40]

Powdered Patties

Powdered substitutes similar to patties, there are dry powdered natural diet substitutes that can be combined with sugar or other attractants like honey and juices. These substitutes are made by combining different types of substitutes[41] like soya powder, yeast, skim milk powder, nectar, vitamins, enzymes and probiotics. These powders can be added to hive feeders by beekeepers.[42]

Sugar Candy

Feed can also be given in the form of sugar candy.[43] It is prepared by dissolving cane sugar and glucose in water by stirring and boiling mixture until temperature of syrup rise to 116 degree Celsius. Then cool the syrup and beat until thick. These sugar candies are given on small strips of wood above cluster of bees by placing an empty supper.[44]

Consumption Rate

Scientists and beekeepers create balanced diets containing carbohydrates, proteins, vitamins, and minerals to replicate the nutritional makeup of natural floral resources. This helps ensure bees receive necessary nutrients during limited natural food supplies. Bees are transitioning to artificial diets due to declining natural food sources due to habitat loss, pesticide use, climate change, and disease spread. So In the university apiary located in Jammu at Chatha, Kumar[38] conducted an experiment to determine the effect of artificial diet on colony build, effect of different artificial diets on the incidence of disease and enemies, and qualitative and qualitative analysis of pollen loads to determine the major floral sources of *A. mellifera*. The study was conducted during a time of shortage of natural food sources, diets are developed to keep bee colonies strong and their honey production going. The outcome showed that diet (Brewer's yeast + gram + skimmed milk powder +sugar + pollen) had the highest net consumption followed by diet (soybean flour + honey + yeast +vitamins). Each of these diets outperformed the control group. Similar study was done by Ullah *et al.*[45] on different colony of *A. mellifera L.* in 2019–2020 summer and winter period and found that the fortified soybean artificial diet was preferred by bees then the date paste and sugarcane syrup. Gemeda[46] conducted the study in all the seasons from September 2011 to June 2013 and found that pea flour has maximum utilized by bees in the rainy and dry seasons. Shehata[47] experimented on two different bee species with two different diets. The research did not discover any appreciable variations in food intake between the Carniolan and Italian colonies during the blossoming and starvation seasons. Colonies fed on diet (Crushed phalaris with Soybean) consumed 91.06 g/week as opposed to diet (crushed panicum with Soybean) 81.84 g/week. Aly[48] conducted the experiment to study the effect of artificial diet in the unfavorable conditions on bee colonies with their replicates on only sugar syrup and found that the soybean diet was preferred by bees over wheat seed and soybean with wheat seed. Ahmad[49] performed the experiment on various bee colonies which are divided into five equal groups and feed upon variety of supplementary diet and found that the soybean diet was maximum consumed by the bee colonies then maize flour and germinated pea flour respectively. Abd El-Wahab[50] conducted a study for a period of 12 weeks on bee colonies and the researchers found that the maximum diet was consumed by bees are formed of (honey +Turmeric and Fenugreek powders + A,D and E vitamins + powdered sugar + orange juice + mint oil + sugar syrup followed by the soybean flour). A comparison in the nutrient composition of the food sources (the quantity of both protein and carbohydrates) and maybe the absorption and being approachable of those nutrients to bee workers impact the amount of broad pollen. These factors also influence by the taste and nutrition of pollen substitutes. As evident from Fig. 1 and Table 1, supplementing artificial diets have positive effect on health parameters of honeybees such as; increased

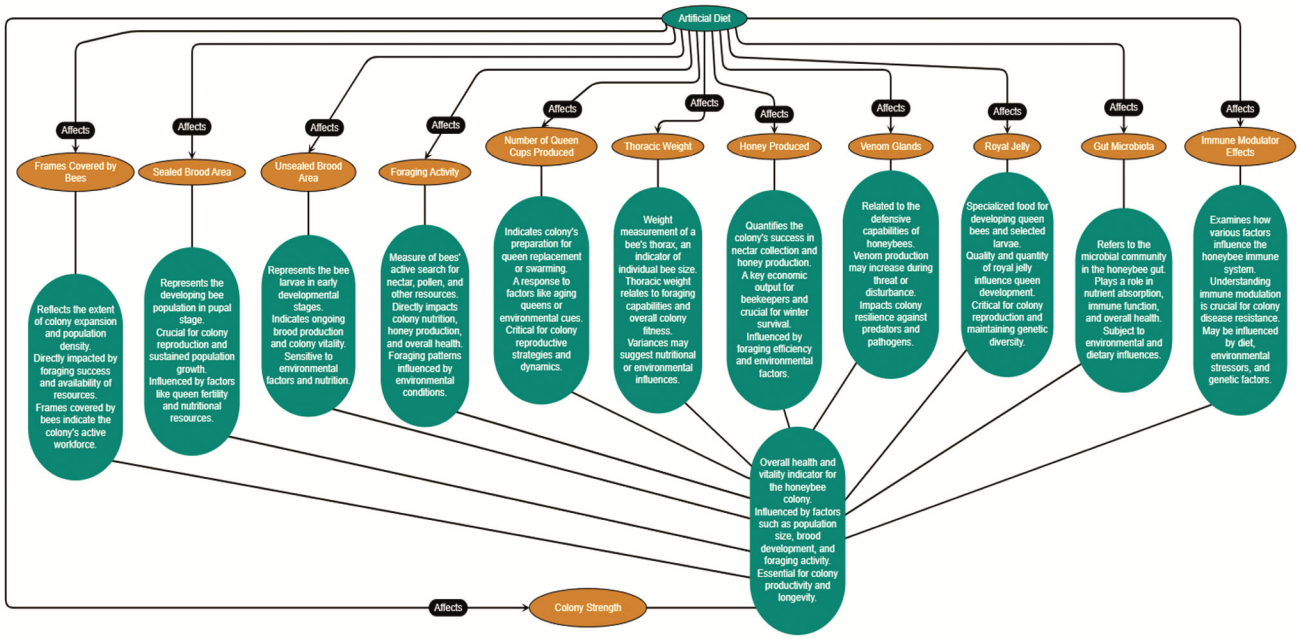

Fig. 1 — Impact of feeding artificial diet on health parameters of honeybees

strength, increased number of brood, enhanced gut microbiota, increased thoracic weight, increased production of enzymatic secretions and immune modulations required for survival of bees during dearth/ unfavourable conditions

Observed Effect on Bee Health

Colony Strength

Especially when it comes to honeybee colonies, bee colony strength plays a critical role in the well-being and output of the colony. It describes the colony's general health, population and capacity to carry out key tasks including defense, breeding, and foraging. Researchers like Kumar[51] studied the strength of bee colony, they found that the bees feed with artificial diet during the dearth period April to September 2010 in the apiary was highest in soybean enriched diet (10.00 bee frames/colony) followed by date fortified food (8.0 bee frames/colony; Table 1). Gemeda[46] also studied the adult bee strength of the colony, he found that adult bee populations also significantly different ($p< 0.05$) with mean strength of 9.7 ± 2.6 frames of bees fed on pea flour compared to 7.3 ± 1.5 frames of bees fed on sugar syrup in same dearth seasons. DeGrandi-Hoffman *et al.*[52] and Taha[53] carried out an investigation on ten colonies of four groups of *Apis mellifera* with four diet and found best strength of bees in diet prepared by brewer's yeast with defatted soybean flour followed by date molasses with defatted soybean flour. The another study was performed by Tomar, and Singh[54] they select the fifty colonies with their five replicates and four are provided with artificial diet with sugar solution and fifth as a control on sugar syrup to perform the experiment and found that the colony given artificial feed with sugar syrup has the greatest strength of bees as compared to the control group only on the sugar feed. Similar study was done by Sihag, and Gupta[55] in Haryana on honeybee colony provided four artificial diets and three were control groups and found that the colonies provided with carbohydrates + protein + vitamins + minerals had the best growth of the colony strength then the other diet and least was observed in control groups. Saffari, Kevan, and Atkinson[56] experimented on 21 colony divided into three groups and control. The colonies were feed on the marketed artificial diet and found that the bees given the artificial diet had the larger population then the bees not provided the artificial diet. Similar studies on bee population was conducted by the Shumkova, Balkanska, and Hristov[57] that the resistance of bee strength by the infection when they are provided with herbal diets. They conducted experiment in two phases and evaluated the bee strength in pre treatment and post treatment after twelve days in rainy and winter season and found that thee herbal supplements had the positive effect on the bee strength of the colony, and the control groups were continuously

Table 1 — Showing preferences of different diets consumed and effect on health parameters of honeybees

S. No. (Ref.)	Number of diet	Composition of diet	Preference of diet according to consumption	Effects
Study 1[38]	Diet 1	Defatted soya flour 20 g + skimmed milk powder 25 g + sugar 5 g + polln 5 g+ glucose 10 g + honey 35 mg	—	Lowest bee strength
	Diet 2	Back gram 20 gm + yeast 20 gm + pollen 20 gm + honey 20 gm + glucose 20 gm	—	Maximum strength of bees
	Diet 3	Brewer's yeast 42 gm + gram 4 gm + skimmed milk powder 4 gm +sugar 50 g + pollen 10 gm PAU diet	Most consumed	
	Diet 4	Soybean flour 60 gm + honey 35 gm + yeast 5 g + vitamins 1g/kg HAU diet	—	Overall brood development-
	Diet 5	Soyabean flour 25 g + yeast 10 g + pollen 15g + skimmed milk powder 5 g + honey 22.5 g + sugar 22.5 g GBPU diet & T diet	—	Minimum brood development
	Diet 6	Control sugar feeding	Least consumed	Inferior to all the diets
Study 2[45]	Diet 1	Soybean flour 30% + skimmed milk 25% + sugar 5% + honey 20% + glucose 20%	Most consumed	Highest worker brood area, bee strength
	Diet 2	Sugarcane syrup 30% + skimmed milk 25% + sugar 5% + honey 20% + glucose 20%	—	—
	Diet 3	Date paste 30% + skimmed milk 25%+ sugar 5% + honey 20% + glucose 20%	—	Highest proportion of foragers
	Diet 4	Black gram flour 30% + skimmed milk 25% + sugar 5% + honey 20% + glucose 20%	—	—
	Diet 5	Grinded groundnut 30% + skimmed milk 25% + sugar 5% + honey 20% + glucose 20%	Least consumed	Least bee strength
	Diet 6	Control (sugar syrup)	—	Inferior to all the diets
Study 3[50]	Diet 1	100 g of diet A (40 g Soybean flour 40 g powdered sugar +100 ml sugar syrup)	Least consumed	—
	Diet 2	100 g of diet B (30 g Soybean flour pollen grains + 40 g powdered sugar + 100 ml sugar syrup)	—	Significant number of queen cups produced
	Diet 3	100 g of diet C (50 g Soybean flour + 25 g honey + 100 ml sugar syrup)	Least consumed	Large amount of honey yield
	Diet 4	100 g of diet D (50 g date pollen grains + 50 g powdered sugar + 100 ml sugar syrup)	—	Highest number of combs covered with bees
	Diet 5	100 g of diet E (10 g Brewer's yeast + 1 g bee honey + 8 g turmeric and fenugreek powders + 0.5 g A, D and E vitamins + 45 g powdered sugar + 20 ml orange juice + 10 ml mint oil + 30 ml sugar syrup)	Most consumed	Highest worker brood area, maximum citrus honey yield
Study 4[51]	Diet 1	Soya flour 16.7% + parched gram 16.7% + Brewer's yeast 16.7% + sugar 33.3% + glucose 16.7%	—	—
	Diet 2	Soya flour 20.7% + Brewer's yeast 20.7% + *Spirulina* 8.3% + sugar 33.3% + glucose 16.7%	—	—
	Diet3	Soya flour 16.7% + Brewer's yeast 16.7% + protein hydrolysate 16.7% + sugar 33.3% + glucose 16.7%	—	Highest sealed brood area, highest egg laying, highest colony population, highest honey storage
	Diet 4	Soya flour 16.7% + Brewer's yeast 16.7% + Protein hydrolysate 8.3% sugar 33.3% + glucose 16.7% + honey 88.3%	—	2nd highs colony population
	Diet 5	Spirulina 16.7%	—	Least sealed brood area, least colony population, least honey storage
	Diet 6	Soya flour 30% + Brewer's yeast 10% + milk powder 10% + sugar 33.3%	—	—

(contd.)

Table 1 — Showing preferences of different diets consumed and effect on health parameters of honeybees (*contd.*)

S. No. (Ref.)	Number of diet (ref.)	Composition of diet	Preference of diet according to consumption	Effects
Study 5[53]	Diet 1	Soya flour (16.7%) + parched gram (16.7%) +Brewer's yeast (16.7%) + sugar (33.3%) + glucose (16.7%)	—	Minimum sealed brood area
	Diet 2	Defatted soya flour (20.7%) + Brewer's yeast (20.7%) + Spirulina (8.3%) + sugar (33.3%) + glucose (16.7%)	—	Minimum sealed brood area
	Diet 3	Defatted soya flour (20.7%) + Brewer's yeast (20.7%) + soy protein hydrolysate (16.7%) + sugar (33.3%) + glucose (16.7%)	—	Maximum sealed brood area, maximum number of frame cover, maximum bee population
	Diet 4	Defatted soya flour (20.7%) + Brewer's yeast (20.7%) + soy protein hydrolysate (8.3%) + sugar (33.3%) + glucose (16.7%) + pollen (8.3%)	—	—
	Diet 5	Spirulina (16.7%) + honey (83.3%)	—	Minimum bee population
	Diet 6	Defatted soy flour (30%) + Brewer's yeast (10%) + Skimmed milk powder (10%) + sugar (50%)	—	—
Study 6[61]	Diet 1	100 g (40 g Soybean flour + 20 g Brewer's yeast + 40 g powdered sugar + 150 ml sugar syrup.	—	—
	Diet 2	100 g (30 g Soybean flour + 15 g Brewer's yeast + 5 g honey + 20 g powdered sugar + 9.5 g powder of fenugreek and turmeric + 20 ml orange juice + 0.5 g A, D and E vitamins + 150 ml sugar syrup	Most consumed	Maximum sealed worker brood area, maximum bee strength, maximum honey yield
	Diet 3	100 g (40 g Maize flour + 20 g Brewer's yeast + 40 g powdered sugar + 150 ml sugar syrup	Least consumed	Minimum bee strength in colony
	Diet 4	100 g (30 g Maize flour + 15 g Brewer's yeast + 5 g honey + 20 g powdered sugar + 9.5 g powder of fenugreek and turmeric + 20 ml orange juice + 0.5 g A, D and E vitamins + 150 ml sugar syrup.	—	—
	Diet 5	Control (1 liter of 50% sugar syrup)	—	Least effective to all diets
	Diet 6	100 g (40 g soybean flour + 20 g Brewer's yeast + 40 g powdered sugar + 150 ml sugar syrup.	—	—
Study 7[62]	Diet 1	Defatted soya flour 23% + skimmed milk powder 27% + sugar 5% + glucose 10% + honey 35% (DSF based diet)	—	—
	Diet 2	Parched red gram powder 26% + skimmed milk powder 24% + sugar 5% + glucose 10% + honey 35% (PRGF based diet)	Most consumed	—
	Diet 3	Parched bengal gram powder 26% + skimmed milk powder 24% + sugar 5% + glucose 10% + honey 35% (PBeGF based diet)	—	—
	Diet 4	Parched black gram powder 26% + skimmed milk powder 24% + sugar 5% + glucose 10% + honey 35% (PBGF based diet)	—	—
	Diet 5	Parched green gram powder 26% + skimmed milk powder 24% + sugar 5% + glucose 10% + honey 35% (PGGF based diet)	—	—
	Diet 6	Parched horse gram powder 26% + skimmed milk powder 24% + sugar 5% + glucose 10% + honey 35% (PHGF based diet)	Least consumed (took longest time to consume)	—
Study 8[69]	Diet 1	Parched gram flour (50%) + stored pollen at 5°C (20%) + glucose (20%) + sugar (10%)	—	—
	Diet 2	Pea flour (50%) + stored pollen at 5°C (20%) + glucose (20%) + sugar (10%)	—	Minimum unsealed brood area

(*contd.*)

Table 1 — Showing preferences of different diets consumed and effect on health parameters of honeybees (*contd.*)

S. No. (Ref.)	Number of diet	Composition of diet	Preference of diet according to consumption	Effects
	Diet 3	Corn flour (50%) + stored pollen at 5 °C (20%) + glucose (20%) + sugar (10%)	—	—
	Diet 4	Parched gram flour (50%) + stored pollen at room temperature (20%) + glucose (20%) + sugar (10%)	—	3rd maximum sealed and unsealed brood area
	Diet 5	Pea flour (50%) + stored pollen at room temperature (20%) + glucose (20%) + sugar (10%)	—	—
	Diet 6	Corn flour (50%) + stored pollen at room temperature (20%) + glucose (20%) + sugar (10%)	—	—
	Diet 7	Parched gram flour (50%) + fresh pollen from flowers (20%) + glucose (20%) + sugar (10%)	—	maximum sealed brood area, maximum unsealed brood area
	Diet 8	Pea flour (50%) + fresh pollen from flowers (20%) + glucose (20%) + sugar (10%)	—	4th maximum sealed and unsealed brood area
	Diet 9	Corn flour (50%) + fresh pollen form flowers (20%) + glucose (20%) + sugar (10%)	—	2nd maximum sealed and unsealed brood area
	Diet 10	Control (without added any diet substitute)	—	Minimum growth of colony

deceasing the bee strength. Singh[58] conducted the study on bee strength which has numerous adult illnesses, ectoparasitic mites, and brood diseases that affected *Apis mellifera* colonies. Nonetheless, colonies fed varying diets continued to be strong, healthy, and resistant to disease and insect attacks. Increased feeding lessened the attack's intensity. The immune response to viral infections is significantly influenced by nutrition, and it is critical to comprehend the relationships that exist between nutrition and personal immunity. The nutritional value of pollen varies greatly, and locations with higher levels of development are linked to higher rates of colony loss.[59] One of the many environmental factors affecting honeybee colonies is bee nutrition (Fig. 1).

Number of Frames Covered by the Bees

When bees are fed an artificial diet that is both nutritionally enough and well balanced, they cannot continue to forage and nurture their young for a considerable number of frames. Even in situations where natural resources are scarce, a high-quality artificial diet can support the colony's population growth, encourage the development of brood, and continue foraging activities.[60] Several research projects are conducted to sustain the quantity of bee frames within a colony under unfavorable circumstances. Kumar[114] found that the colonies fed diets of defatted soya flour with pollen and without pollen had the highest number of frames covered by bees, with respective values of 5.81 in both cases. The control colonies had the fewest number of frames (5.0) covered by bees per colony. Similar study is done by Islam[61] from June to October of 2018, a commercial bee farm. The total number of bee frames in each colony is recorded along with the diets (soybean flour + brewer's yeast + powdered sugar + sugar syrup) and (maize flour + Brewer's yeast + powdered sugar + sugar syrup) had total 9 and 8 bee frames per colony in experimental group then the control. Kumar[38] also studied the effect of diet on the number of frame covered by bees (Table 1). They found that the bees who consume artificial diet had the significant effect on their population and cover the large frame area. The bees feed on the black gram had the largest frame cover in the experiment and lowest was with the soybean flour. Gemeda[46] worked upon the artificial diet and their impact on honeybees using various parameters and he found that the bees under the control using sugar syrup had the low number of frame cover then the bees who consumed the artificial feed made from the pea flour during the unfavourable conditions. Tomar, and Singh[54] worked upon the bee from different apiary and prepared 50 different colony with their 5 replicates and given artificial feed with the sugar solution and found that the bees which were provided with artificial diet and sugar syrup with regular interval of time covered the large number of frames then the bees which are feed on long time duration and also from the control groups. Kishan[62] worked on six artificial diets with three treatments of every diet and worked on the comparison of best diet of bees and found that the increase in number of bees was observed in perched red gram powder then the other diets prepared. Proper nutrient requirement of honeybees will maintain to develop a healthy colony.

The healthy bees can produce more number of eggs and increase their number in the hive and increase their frame covers.As the number of bees increases, it produces larger amount of honey and increases plant pollination (Fig.1 and Table 1).

Sealed Brood Area

The honeybee colony's growth and upkeep depend heavily on the sealed brood chamber. Male bees known as drones are involved in reproduction, while worker bees emerge from these cells prepared to perform a variety of functions within the hive. Worker or drone cells are usually not used for raising queen bees instead; the bigger peanut-shaped queen cells are used.[63] The population of the colony, the resources that are available, and the age and health of the queen bee all influence the size and layout of the sealed brood region in the hive. As part of routine hive management procedures, beekeepers frequently check on the productivity and health of the colony by monitoring the sealed brood compartment. Amro, Omar and Al-Ghamdi[64] conducted a study on honeybee using five protein rich diets as the alternative of pollen diet. The results of this study showed that the control group under natural conditions had the greatest total number of sealed brood cells in the hive and in experimented colonies bee fed with Feed-bee had the mean count of sealed brood area. When adults emerged out, the colonies fed on diet 1 (soybean meal) in bounded conditions were not able to rise the next generation. Similar studies were conducted by Islam[61] with four artificial diets and a control group had the three replicates of each. They conclude from the study that soybean flour and maize reared the largest sealed brood area in the frames and least was observed in control groups. Kumar *et al.*[51] studied the effect of artificial diet in two apiaries located in two different regions in dearth period of summer time. They provide the diet to bees in time interval of 14 days and found the best result for the highest sealed brood area with soybean artificial diet in both the apiaries compared to the control groups (Fig. 1 and Table 1). Khan, Ghramh, and Ahmad[65] also studied the brood area on different colonies having the same eight numbers of frames in each bee hive with four treatments. They found that the bees which were provided with artificial diet of both sucrose solution and locally available pollen substitute have the largest area of sealed brood. The diet was most effective for brood rearing then the control groups. The study of Herbert[66] aimed to figure out the ideal food amounts and how different sterol combinations affected the honeybee brood rearing. According to the study, sealed brood was formed at a much higher rate by bees offered diets enriched with cholesterol or 24-methylenecholesterol than by bees who fed other diets. None of the artificial meals that contained sterols attracted honeybees over the control diet that lacked sterols. Sihag and Gupta[55] prepared several colonies of honeybee with their control groups for the study of diet effect on bee sealed brood area and offered them the five different types of diets to them. The observed results showed that soybean flour with added vitamins and minerals had the best result over all the experimental diets offered and the control group had the lowest results from all. Eissa, Abdelazim, and Abou-Lila[67] in Egypt worked upon the commercially accessible pollen replacement diets for honeybees preparing four artificial diets. The results showed that the brood rearing activity among all the diets was grater in the feed bee. Ashour *et al.*[68] conducted their study with four diets and a control having four experimental groups each. The study found that a liquid yeast diet at 25% confluence resulted in the highest amount of sealed workers brood in honeybee colonies, particularly during spring and summer seasons. Feeding colonies with the liquid yeast diet at 14-day intervals was effective.

Unsealed Brood Area

The continuous development of the honeybee colonies depends on the unsealed brood area. It stands for the area of the hive where nurse bees continuously tend to and care for young larvae. The honeybee's natural life cycle includes the shift from unsealed to sealed brood, which is directly related to the colony's reproductive and maintenance processes. In order to evaluate the health and production of the colony, beekeepers frequently examine both sealed and unsealed brood regions during hive inspections. According to Painkra[69] conducted experiment on the 30 colonies of honeybee during dearth periods and one group was kept as control to which no artificial diet was provided. Parched Gram Flour and Corn Flour showed the maximum unsealed brood area during the unfavorable conditions, Pea Flour showed the minimum result but all are superior to the control. Sihag, and Gupta[55] worked upon artificial diet and prepared four diets for their experimental study and provided them to the four different experimental

treatments. The result shows that the artificial diet of soybean with minerals and vitamins showed the best result on the unsealed brood area in the colony. The diet maintained the colony strength during the unfavorable conditions and provides the necessary nutrients required to make the colony healthy.

Foraging Activity

The act of worker bees departing the hive to find necessary resources for the colony's survival and well-being is known as "foraging activity" in honeybees at peak. During foraging, nectar, pollen, water, and propolis are the main resources that are sought for. Every single one of these resources has a specific function inside the hive, supporting the colony's defense, hydration, and feeding needs. Maintaining this behavior is essential to the hive's overall health, energy needs, and nutritional requirements. In addition to the physical act of gathering resources, foraging is a dynamic process that requires sophisticated communication and navigational techniques to guarantee the effective use of available food sources. According to studies conducted by Ullah *et al.*[45] the impact of prepared foods and the proportion of foragers returning to the hive were found to be significant ($p<0.05$). The data indicated that the largest percentage of foragers (0.25) was found in soybeans, followed by date paste (0.22), sugarcane syrup (0.20), and black gram flour (0.17), while the lowest percentage (0.12) was found in groundnut fed over control (0.09). Pande and Karnatak[70] studied during rainy season of 2007 and 2008 and the results observed at the end of the experiment showed that the highest foraging activity was present in grinded chickpea (27.000 forager/min.), and grinded green gram (24.333 forager/min.) with lowest activity with grinded horse gram and grinded pea (Table 1).

Number of Queen Cups Produced

A bee colony's production of queen cups can vary depending on a variety of factors, such as the colony's overall health and condition, the queen's age and productivity, and the surrounding conditions. The colony produces young queen bees in specific cells known as queen cups. Beekeepers frequently keep an eye on hive conditions and check for the existence of queen cups during routine hive inspections, as the number of queen cups present at any given moment can change. The particular conditions of the colony and the season may have an impact on the precise quantity. If necessary, beekeepers can step in to handle problems with the queen or stop swarming in their hives. Abd El-Wahab[50] experimented for a period of twelve weeks in an apiary. Six groups of tested colonies were created, with three colonies were prepared for each treatment and for control. The quantity of queen cups generated as a result of the several tested experiments compared to control and other evaluated diets, colonies (given Soybean flour pollen grains + powdered sugar + sugar syrup) produced a satisfying number of queen cups compared to control and other tested diets. Ullah *et al.*[45] conducted a similar investigation on the queen cups and discovered that, in a diet prepared with soybeans, the average number of queen cells and the queen cups were superior in contrast, the grinded groundnut diet proved less successful but superior to the control in the case of average queen cells.Ashour[68] studied on various bee colonies using four artificial prepared nutritive diet and the results of study reveal that mean number of queen cells were produced in tested bee colonies which are feed with different pollen replacement and sugar syrup and in control group at the time of summer and spring time. The highest production of queen cups was produced in dried brewer’s yeast with other nutritional required components. Abd El-Wahab *et al.*[50] studied the effect of artificial diet on the biological activity of honeybees. They prepared five pollen substitute diet for their experiment on equal number of bees in each colony having the four frames per colony. The result observed was highest in the diet of Soybean flour + brewer’s yeast + date pollen grains +powdered sugar + sugar syrup for the number of queen cells covered in the colony over three other diets which were experimented (Fig. 1 and Table 1).

Thoracic Weight

The bees' thoracic weight is very important since it affects their capacity to perform vital tasks for the colony. The flight muscles, which are located in the thorax, are what determine a bee's ability to navigate, forage, and produce more honey overall for the colony. For effective flying, worker bees, which are in charge of gathering nectar and pollen, depend on their thoracic strength. Furthermore, drone mating behavior is heavily influenced by the thoracic weight, which affects the drones' capacity to pursue and mate with queens during their nuptial flights.[71] At the end of the day, a bee's thoracic weight plays a crucial part in determining its physical state and usefulness inside

the hive. To study the thoracic weight Ullah *et al.*[45] conducted a series of experiments on bee colonies using various diets, and discovered that the worker bee's thoracic weight reached its highest in soybean flour and date paste (33.12 and 32.54 mg/bee), whereas the control group had the lowest weight value (23.17 mg/bee). Kim[72] studied on thoracic weight and dried head weight of honeybees using four artificial diets and feed them to the different colonies in the e experimental setup in different combinations to the bees. They observe the result that the honeybees feed on the diet defeated soya bean diet had the highest thoracic weight as compared to the control and other experimental diet. Hendriksma, Pachow and Nieh[73] worked on honeybee weight using different types of amino acid like essential amino acid or non essential amino acid with carbohydrates, protein and different dietary nutrients on honeybee colonies on caged and foraged bees and observed the results that the essential amino acid had the significant effect on the weight of bees. The bees which utilize the essential amino acid had the highest thoracic weight of nurse bees in the colonies. De Souza, Huang and Tarpy[74] experimented on the quality of the queen bees using the artificial pollen substitute diet in high quality colony and low quality colonies. They reared the colony by providing the diet to queen bees and observed the result in new emerged queen bees and open mated queen colonies and found that bees had the increased thoracic weight in both the conditions when they are feed on the artificial pollen substitute diet. Abd El-Wahab, and Gomaa[75] also performed the experiment on the honeybee colony with artificial diet to find out the result on the queen cups production in the hive. They formed nine artificial diets and experimented them on twenty seven bee colonies and observed that colonies feed on 50 percent yeast culture produce highest number of queen cells followed by the 25 % of yeast culture and all the experiments have the good results than the control groups (Fig. 1).

Honey Produced

The bees' ability to produce honey is crucial for both the environment and the economy. In terms of ecology, honeybees are vital to pollinate a wide variety of plant species, including many foods that are necessary for human sustenance. Ecosystem biodiversity is greatly enhanced by honeybee pollination. The production of honey is a significant global enterprise from an economic standpoint. Honey has multiple uses as a natural food preservative; sweetener and ingredient in different goods.[76] In addition to providing revenue for beekeepers, beekeeping and honey production boost rural economies. Furthermore, by offering ecological services that go beyond the production of honey, honeybees help maintain the balance of natural ecosystems, which is why their role is essential to both the global environment and human livelihoods. For the betterment of production on honey several studies are done on bees by researchers now days. The research for a 12-week period found that the colonies fed a new artificial diet formed of brewer's yeast + bee honey + Turmeric and Fenugreek powders + A, D, and E vitamins + powdered sugar + orange juice + mint oil + sugar syrup), produced a significantly higher amount of citrus honey (3.7 kg/colony) in comparison with control colonies. Kumar and Agrawal[77] experimented on honeybee colonies with artificial diet to observe the amount of honey produced in two different apiaries and found that the artificial diet had significant impact on honey production. The colonies which consumed artificial diet produced more amounts of honey then control groups. Gemeda[46] also conducted the experiment to check the effect of diet on honey production and prepared two treatments with number colonies. He observed that the colonies with diet of pea flour produced more honey then the colonies which are provided only the sugar syrup. Tesfaye[78] conducted the studies on different diet compositions with equal number of frames for the treatment. The highest honey store was observed in the colonies that were kept on soybean feed and the least amount of honey was observed in the sugar syrups colonies. Islam[61] carried out a similar investigation. The results indicate that the colonies fed an artificial diet of soya flour and maize flour produced the highest honey yield (9.2 kg/colony), while the control colonies that were not given pollen substitute diets produced the lowest honey yield (4.3 kg/colony). Ahmad[49] also examined the dietary effect of artificial feed to bees in number of colonies and observed that the soybean flour with other essential and nutrient components show the high honey production in the bee hives. Shehata[47] discovered that, during the era of food scarcity, honeybee colonies fed artificial diets differed significantly in their honey yields, and that the production of honey declines as the amount of pollen in diets diminishes. Colonies fed a mixture of sugar

syrup and protein supplement yielded higher honey production. More pollen replacement was consumed, which increased the amount of honey and brood produced. The colonies fed specially prepared diets had greater quantities of honey storage, indicating that the worker bees gathered more nectar, which in turn produced a higher yield of honey (Fig. 1 and Table 1).

Venom Gland

Venom production and the functionality of venom glands in bees are crucial for defense mechanisms and survival. These glands produce venom containing complex biochemical compounds that serve as powerful preventive agent against predators or competitors.[79] The efficiency and health of venom glands are important for the bee's ability to effectively development of venom when threatened, ensuring its protection and territorial safety. Understanding venom production and gland functionality also impart important role for various applications in fields such as medicine, pest control and biotechnology. Various studies on venom glands and its production were carried out with several artificial diets by researchers like Nowar[72] with three treatments of fifteen colonies on three diets in two different experiments and observed that the size of venom sac and venom gland of bees were increased which were provided with artificial diet. The production of venom was also increased. Nowar[80,81] also carried out similar studies on bee venom sac and found that the highest venom sac volume was observed in bees when fed on bean mixed pollen with clover diets followed by maize and pollen diets. Least was observed in sugar solution compared to others. Ibrahim, Mahfouz and ElBassiony[82,83] studied in different seasons to analyze the effect of protein diet nutrition on bee venom weight. The results showed that the pollen grain diet increased the bee venom during the spring season, during the summer and autumn season the highest bee venom was recorded in pollen feed while the control groups had the lowest venom production weight in all the seasons compared to control. Badawy[75] experimented on three groups of honeybee with different protein substitutes and found that the pollen feed had the highest dry bee venom collection from the bee colonies, El-Shaarawy *et al.* [84] also found the best results of highest venom produced with pollen grains followed with milk powder than medical yeast and solution of sugar give their results respectively (Fig. 1).

Royal Jelly

Royal jelly is crucial for bee colonies as it is the main food component for developing queen bee larvae, control their proper growth and reproductive capability.[85] Its rich nutritional content, including proteins, vitamins, and hormones, contributes to the queen's longevity and fertility. The presence of royal jelly determines the social structure of the hive, causing the organization and productivity of the colony. The investigation by Zaytoon, Matsuka and Sasaki[86] on bee colonies with three diets revealed that the royal jelly production in the colony feed with the diet formed by soya protein showed the best results in both brood area feeding method and top bar feeding method as compared to all the treatments. El-Dien[87] worked on honeybee with three formulated diets and the study found that colonies fed on soybean with date paste and horse gram with check pea had the highest production of royal jelly. All the formulated diets showed the highest results as compared to control groups during the unfavorable conditions. Wang[88] worked on the quality of royal jelly and found that the sucrose based artificial diet produced the royal jelly with increased amount of fructose content. There is no significant difference in the unit production of royal jelly .Without the royal jelly the survival and success of the bee community would be significantly compromised. Kanelis[81] also found that the artificial feeding impact the quality of the royal jelly by carbohydrate composition. The sugar feeding alters the glucose sucrose and fructose content in the royal jelly. The colonies feed artificially produced more sucrose in the colony similar to sugar syrup. Oskay and Bayrak[89] investigated on the royal jelly production with the feeding substitutes to bees and found that the bees provided with artificial diet had the increased production of royal jelly with suitable pH and increased protein content in the jelly. Artificial pollen substitutes are beneficial for the quality and quantity of the royal jelly (Fig. 1).

Gut Microbiota

The gut microbiota of honeybees consists of diverse range of bacteria, fungi and other microorganism. They are essential for the bee's digestive health and overall well-being. These all symbiotic organisms assist in the breakdown of complex sugars found in nectar and pollen, help in nutrient absorption. Moreover, they play a crucial role in protecting bees against harmful pathogens by

competing for resources and producing antimicrobial compounds.[90] The presence of gut microbiota in honeybee's colonies was examined with relation of several artificial diets by Ricigliano, Williams and Oliver.[91] *Lactobacillus* Firm 5, *Lactobacillus* Firm 4 and *Bifidobacterium* abundance were highly influenced by the apiary site. These taxa's maximum abundances were seen in colonies at apiary site. Powell[92] compared the POL (control pollen feed bees) group to the bees receiving the artificial diet, the POL group had a lower absolute bacterial population. When a protein substitute was used instead of pollen-fed bees, the gut micro biome had less species diversity and regularity. As determined by effective species number (ESN), bees in the artificial diet added with hemi-cellulose and pectin and artificial diet provided groups had reduced alpha diversity in comparison to POL bees. D'alvise[93] also conducted studies on gut microbiota and found the variability in gut bacterial communities in different seasons, with *G. apicola* fractions being more consonant in summer samples and *lactobacilli* fractions more uniform in winter period. There was a seasonal difference in level of *N. ceranae*. It was detected in all bees in summer season and only in 9 of 45 bees in winter. The composition of the bacterial communities in late winter samples was not significantly different between experimental groups. The number of *Rhizobiales* and *Bifidobacteri*a were reduced in colonies fed with sucrose syrup as a winter feed. Topal[94] studied the microbiota of bees and found that the overall microorganism load was higher in honeybees which were fed with mixed pollen, different syrup and commercial bee cake. Nurse bees showed an increase in microorganism load with time. The highest microorganism developments were observed in honeybee colonies fed with poppy pollen but the load decreased in starting of spring. In fields, bees which were provided with commercial bee cake and syrup showed an increase in intestinal microorganism over time. The species present in intestine were *P. agglomerans and P. luteola.* Kim[72] analyze the micro biome profile of bee's guts with artificial diet and found that Bacilli, *Alpha roteobacteria, Gamma proteobacteria and Bifidobacterium* were commonly present in different diet groups. *Alpha proteobacteria,* was present in syrup diets in abundance. Lactobacillus was most abundant in all diet groups; *Rhizobiaceae* was highest in syrups and abundant in beebread diet groups of honey (Fig. 1).

Immune Modulator Effects

Artificial diet's primary role to provide essential nutrients and their immune-modulatory effects on bee's health are of special interest. Several studies suggest that some artificial diets may influence the response of immune system of bees, possibly affecting their resistivity to pathogens and parasites. Components such as specific amino acids, vitamins, and phytochemicals are presented in these diets which interact with the bees' immune system. They either improve or compromising their ability to fight infections. Borges, Guzman-Novoa and Goodwin[95] provide the supplements in the sugar syrup to monitor the nutraceuticals and immuno stimulatory compounds affect the spore multiplication and mortality of bees at the time of *N. ceranae* infection in colony. Results found that the thymol in diet was the supper effective. Sulforaphane mixed in sugar syrup in different concentration was the most effective; at low concentration it reduces the spore formation but at high concentration stops the spore formation activity of fungi and bacteria in the colonies. Similar studies were conducted by Glavinic[96] observed that by the artificially formulated diets reduced the spore formation capacity of *N. ceranae* in the bee colonies. DeGrandi-Hoffman[97] studied the DWV (deformed wing virus) in bees providing the artificial diet and observed that the bees which feed with pollen or protein substitute diet had the low number of virus carrying bees in the hives. Difference in the DWV is observed on which type of feed they consumed. Stanimirovic[98] observed effects of supplementary diet on different virus in bee colonies and found that ABPV (Acute Bee Paralysis Virus), CBPV (Colony bee Paralysis Virus), SBV (Sealed Brood Virus) and DWV were decreased in colony by use of marketed supplement provided to bees gradually according to the time. Braglia[99] observed the positive results with increase acetic acid and p-coumaric acid amount in pollen substitute diet decreases the *N. ceranae* infection in the honeybee colonies during the winter season (Fig.1).

Gene Expression

Variations in diet's composition, for example changes in nutrient ratio or deficiencies in important nutrients which can activate modification in the expression of genes participating in metabolism, immunity, detoxification, and other crucial biological functions. Suboptimal diets may disrupt the fine

balance of gene expression patterns, possibly leading to physiological stress, impaired immune responses, and reduced overall fitness in honeybee colonies. To study the gene expression Wheeler and Robinson[100] studied on fat body gene expression and observed honey feed bees have 104 and sucrose fed bees had a total of 220 genes differently expressed gene. Study also supports the similarities across the list of genes, with honey increased amino acid, metabolism and oxidation reduction and sucrose up regulates genes which are responsible for axonogenesis, organ development and anion transport. Mao, Schuler and Berenbaum[101] worked on semi artificial diet with and without p-coumaric acid and observed that organ size is influenced by p-coumaric acid which alters the expression of 26 genes from the overall 46 genes. The positive regulation of different xenobiotic metabolizing cytochrome P450 genes and genes responsible for immunity were observed which was same with those positively regulated by p-coumaric acid in adult. Danihlík *et al.*[102] observed no significant difference between the pollen diet and no pollen diet. They also observed the increased concentration of apidaecin 1 isoformis gene expression in thoraces of honeybee with protein diet when compared to diet without pollen. According to the study of Wang *et al.*[103] foraging choice of worker bees was influenced by IRS (*insulin receptor substrate)* expression but not influenced the sucrose response system. This might be due to IRS had well-defined roles in behavioral control vitellogenin. According to the observations, there was no whole effect of IRS RNAi on the quantity of vitellogenin transcript. But vitellogenin expression was mostly decreased in low pollen-hoarding strain bees following IRS RNAi, the process was not observed in high strain worker bees. The study of Nilsen[104] revealed that ilp1 and ilp2 mRNA levels in fat body cells are separately expressed. The level of ilp1 increasing powerfully in effect to BSA injection but ilp2 expression remains uninfluenced by vg (vitellogenin) suppression. Quinlan[105] also found differences in gene expression among diets for JHamt, Vg, Ilp1, and ILP-2, with higher expression of Vg and ILP-2 among bees from sucrose syrup-fed colonies. Fengkui[106] found increased expression of TPH gene , 5-HT1R gene, 5-HT2βR gene expression and 5-HT7R gene expression when bees are treated with different dietary substitutes, also the study of Negri[107] revealed that to 1118 W gene, Nos gene and ppoact gene levels increased significantly with artificial diet when compared to control and in cold stressed bees also observed that bees provided with invert sugar and high fructose corn syrup down regulated the vitellogenin and ILP-2 genes expression but up regulated the expression of JHamt and ILP-2 genes expression in bees which overwintered on these diets and which develop in early spring (Fig. 1).

Survival Rates

The composition of artificial diets directly impacts bee health and their survival. A well-balanced artificial diet can provide essential nutrients required for bee development and immune function, potentially leading to improved survival rates. Imbalances in artificial diets may result in nutritional deficiencies, weakened immune systems and at last decreased survival rates among honeybee colonies. Thus, careful formulation and consideration of artificial diets are vital for supporting honeybee populations, particularly in the face of challenges such as habitat loss, pesticide exposure, and disease pressures. Found the bee consumed different artificial diets had different survival rate. The bees provided with fructose corn syrup, sucrose syrup, invert syrup had the less survival rates compared to honey during the winter season. Invert sugar and sucrose syrup had good results over all experimental groups. Oskay[108] studied the effect of diet with survival rates of bees and concluded that solid feed diet with 10% protein substitute (mixture of inactive bread yeast extract powder, powdered beet sugar, inverted sugar syrup (70% sugar, 30% water), sunflower honey and sunflower seed oil) showed the best survival of bees. Kaftanoglu, Linksvayer and Page[109] observed adult and larva survival rates of *A. mellifera* larvae which revealed that larvae may develop without use of carbohydrates but cannot pupate or become adults without required amount of carbohydrates. Survival rates of bees increased when diets contained 12% sugar were used and glucose and fructose ratios were not crucial. The study of Arien[110] found that bee survival was significantly affected by the lipid concentration in the diet. Bees fed with 4% lipids had the highest survival chances followed by 8%, 2%, 4.7% (pollen) and 1% lipid diets. The omega also affected the survival rates, with the high ratio having the lowest survival. Bouchebti, Wright and Shafir[111] also found that the protein to carbohydrate ratio affects the survival ratio of honeybees. Greatest survival was found in ratio of 1:19 of P:C. Excess

protein in diet can reduce the survival rate. According to Yazlovytska[112] control group had the highest survival rate in the experiment, while those bees consuming sucrose solution with oil seed (rapeseed) or willow pollen had highest mortality rates. The sugar solution supplementation with willow beebread, willow pollen or rapeseed pollen found the highest mortality rates. Fengkui[106] studies found that lifespan of worker bees were shortened under controlled conditions when they were feed with dietary supplements in all groups decreasing as days increased. Worker bees fed with 12mg/g tryptophan had the highest survival rates in bee colonies (Fig. 1).

Health and Diseases of Honeybee

The construct or choice of diet can affect an organism's condition to pathogens, and this is especially observable in honeybees. Pollen and nectar or honey holds in various phytochemicals and micro nutrients that can affect the immune response of honeybees. Various diets are likely to provide bees with the necessary nutrients and higher pollen diversity which has been shown to up regulate some effects of the innate immune system and reduce mortality of bees due to pathogens like *N. ceranae* and IAPV.[113] Nutrition and quality of diet affect Nosema levels in bees, studies showing that providing honeybees with polyfloral pollen increases immune-related enzyme's activities, making colonies more resistant to stress.[114] honeybees are susceptible to many diseases like Nosema, which can decrease their population size during winter months. Singh[114] found that the colonies given different diets maintained bee strength, health and resistance against pest attacks. Supplemental feeding can reduced attack intensity in various brood diseases include ABF (American foul brood), EFB (Europian Foul brood), SBV (sac brood diseases) and adult bee diseases like Nosema diseases, some species of ectoparasitic mites. Similar results were corroborated from previous studies.[108–111] Colonies given PS exhibited the highest frequency of DWV and BQCV and little to no effect on DWV levels. Kumar[115] recorded the samples of larvae, pupae, and adults from each colony and debris and analyzed them for mites and related pathogens. Honeybee samples were examined for mite presence and combs were examined for wax moths revealed positive impact of diet in colony. Ricigliano, Williams and Oliver[91] also found the ration of *N. ceranae* with the use of supplementary feeding in the colony. The infection can be controlled by providing them he substitutes. Braglia[99] also observed the supplementation of Phytochemical in bees infected with *N. ceranae* importantly affected spore load at mortality. Caffeine, kaempferol, and p-coumaric acid can reduced spore-loads at the low concentration was most effective. The supplementation also enhanced normal longevity of infected bees at low or medium concentrations (Fig.1 and Table 1).

Gap in Studies

The studies on artificial diet and their effects on honeybee's health has been conducted from several years, however there are several gaps in the studies make the relationships between the research difficult to interpret. Firstly, there is a need to study the long term ecological impact of artificial diet on bee population and broader ecosystem. Next, there is a limited study which reveals the information about the bee behavior like defense, hygiene, temperature regulation, nursing the brood and foraging. There are less evidences about influence of diet on gut microbiota and the susceptibility to pathogens.

There is a need for research into the optimal nutritional composition of artificial diets for different bee species and environmental conditions. Developing customized diets that meet bees' nutritional requirements can enhance their health and productivity. There is less investigation about the relation of artificial diet and the pest management in bee keeping practices. There are studies only about the domesticated bees and there are no results for the wild bees and their interaction with the native plants which bees utilize for their requirements. Addressing these gaps through future researches are important for developing sustainable and effective artificial feeding practices that support bee health, ecosystem resilience, and food security.

Future Perspectives

Artificial or supplementary feeding to bees has been practiced by researchers and beekeepers for a long period of time particularly during the times of dearth or in unfavorable conditions or the scarcity of natural flora to bees to prevent the harm caused to bees. There are many considerations for the future of artificial feeding to maintain the beekeeping. Artificial diet can mimic the nutritional requirement of bees in adverse conditions. It can provide them the

essential nutrition to maintain their colony. Artificial feeding can provide the essential medications to the bees to prevent diseases or medication can be added to the bees' diet to treat the bee colonies suffering from diseases or any particular nutrient deficiency. The formulated diet can have the specific component or nutritional content required by the bees at the different time of the year and according to their local environment. This might involve adjusting the nutritional content at specific conditions. It is also essential to consider the environmental impact on the artificial diet of bees along with the human intervention. Researchers and beekeepers must evaluate the possible ecological effects of artificial feeding practices become common and take steps to reduce any unfavorable effects on natural ecosystems. Advance precision feeding method could allow beekeepers to provide the artificial feed to bees more accurately and efficiently. This precise method could help to minimize waste and right amount of supplement food at right times. The continuous studies on supplementary feeding to bees insight in the long term effect of artificial feeding on bee's health, their productivity and behavior and make researchers or beekeepers informed about when and how to feed their colonies diet. The future development and the artificial diet should be aligned in the principle of sustainable development. This might include the usage of eco-friendly feed ingredients, minimize the use of added chemicals or preservatives and consider the overall ecological footmark of the supplementary feeding.

Conclusions

Honeybees supplementing with artificial diets offers a possible way to improve bee health and maintain the long-term viability of bee populations. Our analysis presents the beneficial effects of several supplemental diet on the nutrition, immunity, and general health of honeybees. These results highlight the potential benefits of such treatments in reducing the negative impacts of pesticide exposure and habitat loss, among other environmental stresses. Nevertheless, there is a need for further specialized study because the efficacy of these diets might differ depending on composition, bee species, and environmental factors. Future research need to concentrate on evaluating ecological compatibility; understand long-term effects, and improving diet formulas. Furthermore, extended field testing is necessary to confirm lab results and modify real-world implementations. We can boost resilience by increase our understanding and use of artificial diets.

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