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Catalysation of Artificial Sourcing on Honey Bees: The Direct Imprint on Physiological Processes and Gut Microbiota

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Microbes in the insect gut, forming the insect gut micro biome, include bacteria, archaea, fungi, and protists, and they are vital for insect survival, performing roles in digestion, nutrient synthesis, immune defence, detoxification, and development. These microbial communities are shaped by environmental habitat, diet, and the host insect's phylogeny, with bacteria like Proteobacteria and

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Firmicutes being dominant in many insect guts. The interactions are often symbiotic, with insects providing a stable environment and nutrients, while microbes provide benefits like producing vitamins and breaking down plant toxins. For the microbial inhabitation inside the body, insects gut is enormously flooded with distinctive environments in which one entity of microbial world called bacteria provides many indispensable services to the insect. To perform the vital functions, insect body exhibit a wide range of dependence on the gut fauna especially the bacterial population. In dearth and lean period insect digestive tracts vary extensively in morphology and physicochemical properties, factors that greatly influence microbial community structure. The artificial sourcing of the honey bees in terms of essential and non essential amino acids such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine has been documented with upto the mark vital functions in the insect body especially the amino acid leucine. The disease controlling ability and nutrition supplement were thoroughly exploited by artificial diets incorporation in apiary by human involvement. The insufficient nutritional deficit in the area were compensated by routinuous feeding of artificial diet “pollen substitute”.

Keywords: Bacteria; population; amino acids; apiary; artificial diet.

1. INTRODUCTION

As far as biomass and ecological richness globally is concerned insects are the most abundant animal group in the universe (Basset et al., 2012). The relationships and interlinked pattern of living with beneficial microorganisms, had led to the wide diversification and evolutionary success of insects which are known to enhance the quality of nutritionally meager diets, accelerating the digestion of recalcitrant food components, and many other inter and intraspecific communications (Holt et al.,2024). The major breakthrough in the production of the honey and the colonies occurs primarily during the inclement weather conditions especially in winter regardless of climate. Moreover, overwintering in temperate regions has been deliberated (Mattila et al., 2001, Steinmann et al., 2015, Smedal et al., 2009, Guzman-Novoa et al., 2010) comparatively minimal physiochemical and behavioural responses from honey bees overwintering in climates of the different regions. Due to the very short duration of winter months and relatively high temperature the growth of honey bees may get continue, but the constraints and discontinuous brood rearing and foraging activity made the resources scanty when prolonged duration of forage dearth and abrupt jolts of disconsolate temperatures go hand in hand. The poor sub-standard dietary supply, immune dysfunction, inability of the queen to grow and the increases number of different predators and parasites, more or less are the exclusive factors responsible for the poor colony development. (Dhruba, 2009, Gallant et al., 2014, Vanengelsdorp et al., 2008, Simone-Finstrom et al*.,* 2016). Most predominantly vitellogenin, a nutritionally-regulated storage protein molecule on which the management of the colony in terms of nutrition and production relies, is highly seen during the fall winter months. Being the most bumptious molecule, it elevates the tolerance to the starvation and oxidative stress thus overall increase the longevity of the honey bees. In order to suffice the pollen supply when the foraging is below the desired level in high-density apiaries, the honey bee colonies are typically fed artificial diets blended with pollen substitute (Mattila & Otis 2006, de Jong et al., 2015, DeGrandi-Hoffman, et al. 2008). It has been revealed that not all the artificial diets are upto the mark of natural pollen (DeGrandi-Hoffman & Chen 2015, Al-Ghamdi et al*.*, 2011, DeGrandi-Hoffman et al., 2010) and could greatly influence the activity of the biomolecules and kinetics of enzymatic reactions that sways the physiology of honey bees beyond their primary nutritional roles (Mao et al., 2013, Pyrzynska & Biesaga, 2009, Mao et al., 2015, Ricigliano et al*.,* 2015, Wheeler & Robinson, 2014, Wilson et al., 2013). The best possible way of artificial sourcing could be the incorporation of the constituents present in the natural pollen diet or components of it which on fermentation by the honey bee gut bacteria results in molecular signaling to honey bee metabolism and aging (Lee et al., 2015, Engel & Moran, 2013, Zheng et al., 2016). The micro biome dynamics, pollen exhaustion and physiology efficiently modulate the expression of immune genes, draws an attention towards the fact that the essential prebiotic fingerprints of pollen supplement could play a great role in modulating the immunity.

2. GUT OF HONEY BEE INSECT

The insect digestive tract resembles greatly associated with adaptation to different feeding modes. The insect gut is endowed with three primary distinctive regions, foregut, midgut (or ventriculus) and hindgut (Chapman et al., 2013). The embryonal ectoderm which form the exoskeleton of foregut and hindgut is made up of chitin and other cuticular glycoproteins and enzymes. During the ecdysis the exoskeleton is separated from the gut lumen. For temporary food storage, the foregut often has separate crop or diverticula and the hindgut encompass the fermentation chambers and defecation organ in the form of rectum. For efficient digestion and absorption of the materials, midgut plays an important role in many insects is and so in the diverse pollinators which have chewing and lapping type of mouth parts; an endodermal in origin and lacks the exoskeletal lining (Engel & Moran 2013).

3. POLLINATION EFFICIENCY

Amongst the honey producing bees, the *Apidae* family is most quintessential pollinators in agricultural crop ecosystem that are not only cultivated for their immense honey producing capability and byproducts but, majorly contribute to the biodiversity sustainability of an ecosystem (Klein *et al*., 2007; Gallai et al., 2009). In order to maximise the brood production, excellent immune function and overwintering percentage, the abundance of floral resources plays a pivotal role. The optimum source of carbohydrate is supplied by nectar foraging along with pollen material which is a unique source of proteins, vitamins, lipids, and micronutrients. The honey prepared from natural foraging is sticky solution of various ingredients such as carbohydrates (>80 per cent), water (15-20%), protein (< 1%) and contain the minute concentrations of different amino acids, vitamins, enzymes, phenolics and other antioxidants (James, 2009).

Standifer et al., (1977) analysed that the ample amount of nourishment given to the larva and adult is majorly by carbohydrates from pollen, certain fruit and plant juices which maneuver a normal growth and development of the honey bees such as efficient working and functioning of multiple organs such as gonads which easily help in the heat dissipation from the body and muscle movement, the ample wax production at an optimum quantity being the remarkable one. Nevertheless, the mature bees flourish successfully on the variety of the carbohydrate source such as glucose, fructose, sucrose, trehalose but seize to show any kind of glucose units consumption such as lactose, raffinose, dextrin, galactose, mannose, xylulose and inulin. Furthermore, the studies done by various scientists elucidate that lipids (sterols and fats) mediate a minute role in synthesis, of glycogen and body functions; however, scientific investigations have not conclusively put forward whether bees are in need of fatty acids in their diets. The bee fauna requires and utilizes some of the lipid content in their early growth stages of life, present in the pollens they consume. Though, dietary sterol has been found with phenomenal role in most of the insect groups especially in the gut lubrication for efficient ingestion till the food is digested and assimilated (Tritschler et al., 2017). The continuous protein supply and definite amino acid composition for optimum nourishment of the young adult bees and for brood food-producing hypo pharyngeal glands of nurse worker bees is also advantageous (Crailsheim 1990).

The huge downfall in Indian beekeeping industry is to withstand with the harsh conditions for maintaining ample strength of honeybee colonies in which pollen supply and honey is no more for feeding (Mayack, & Naug 2009). Due to the frequent inclement weather especially in winters and summers where temperature fluctuates below or above sub-optimal level in temperate and tropical regions bee flora is inaccessible. The scantiness of the bee diet is aggravated in several parts of our country where dearth periods take a bit longer as compared to other parts which leads to the dwindling eye vision and even death of bee colonies. Thus greater care is required in management of apiary (DeGrandi-Hoffman et al., 2008, Sihag & Gupta, 2011). Despite the number of options available, the most convenient is migration of honey bee colonies but it require lots of labour, time, money, and also exposing them to a variety of biotic and abiotic stressors, and the other option being is to feed the bee colonies with rich supplements of artificial diet so that the process of brood rearing can be enhanced and strength of colonies is maintained (Saffari et al., 2006, Chmielinski, & Grozinger 2021). Scientists have come up with the diverse artificial diets and their effect on various colony parameters were observed (Hendriksma *et al.,* 2011a). The number of other sophisticated methods have also been devised through tedious processes to rear honey bee larvae in the laboratory by revamping the dietary needs, in which royal jelly was diluted with an aqueous solution of glucose along with fructose. Hendriksma *et al.* (2011b) also opined that the importance of fungi as a substrate for nutrition such as yeast in the artificial sourcing for larvae of several close groups, but was replaced with vitamin B and polyunsaturated fatty acids in later stage. The unicellular fungi such as yeast were also employed for artificial dietary source to the larvae population, (Aupinel et al., 2005) but was later on exempted by several researchers without a reduction in individual survival rates (Genersch et al., 2005; Genersch et al., 2006). The yeast has been also habituated as differentiating agent for female castes, due to the phagostimulatory or nourishing effects (Aizen & Harder 2009). The readily available vitamin formulation (Moritz et al., 2010) was also accustomed apart from the yeast using as a nutritional source for enriching larval diet, which did not affect larval weight or mortality significantly (Cilia et al.,2021).

Keeping the losses of the bee industry into consideration due to the unprecedented bad colony performance, in this review effect of various diet formulations fed to bee colonies during dearth periods have been assessed to measure the colony parameters like brood rearing, bee strength, number of bee covered frames and its impact on microbes present in gut of the honey bees so that suitable sourcing of food and pollen substitute can be developed to improve beekeeping practice globally.

4. LITERATURE REVIEW

For the different vital functions to happen such as honey bee brood production, immune responsiveness, and survival over harsh conditions, the ample amount of flora and diversity is of the paramount importance (Di Pasquale et al., 2016, Meikle *et al*., 2017). Due to the indispensible use of agrochemicals, genetically modifies crops and pest resurgences in colonies, the number of sub-lethal effects were comprehended which lead to impaired immune function, reduction in longevity and very minimal brood production (Vodovnik et al.,2021, Hofman & Chen 2015, Koch et al., 2017). The pollen fidelity and varied flower sources was very essential for various reasons such as stimulation of colony growth, accelerated brood formation, to meet all nutritional requirements and resilience to biotic and abiotic factors as it being the rich source of proteins, lipids, and micronutrients (Brodschneider & Crailsheim 2010). Amongst the different constituents in pollen, the most vital functions by the bees have been found immensely upto the mark by the proteinaceous content of pollen diet in particular, the amino acids essential as well as non-essential such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (De Groot, 1953). Pollen also contains essential lipids that are important to various aspects of bee physiology (Avni et al., 2014, Arien et al., 2015, Wegener et al., 2018, Robinson & Nation 1966).

The poor and tempestuous weather leads to the poor foraging conditions and intensive cultivated landscapes have lead to grave threat to bee nutrition. (Roulston et al., 2000, Roulston & Cane 2000, Donkersley et al., 2014, Sponsler & Johnson 2015, Dolezal et al., 2016). Many beekeepers across the globe feed artifcial “pollen substitute” diets in dearth periods when the supply of pollen is insufficient and to accelerate the colony strength before rendering them for pollination (Mortensen et al., 2019, Lamontagne-Drolet et al., 2019, Mattila & Otis 2006, Noordyke & Ellis, 2021). It has been opined that more than 80% of beekeeper farmers claim to feed artificial formulated diet throughout the United States (Bee Informed Partnership, National Management Survey. The man made ingredients have been employed for feeding purpose in order to replace natural pollen so that the pollen requisites are sufficient. The artificial ingredients with sumptuous protein-rich constituent entrenched with soya, pea, yeast, casein, egg, and microalgae were some diets rich in fraction of bee-collected pollen, which has been registered in positive correlation with consumption and brood rearing (Standifer et al.,1973, Alqarni, 2006). For the nutrition of the young worker bees the feed were placed inside the hive for consumption and nutrition reservoirs were build up that are used to rear brood. The widely used parameters for the quantification of diet efficacy are population size, strength and worker bee physiology.

5. GUT MICROBIOTA ABUNDANCE

Owing to the immense distinctiveness of the insecta class, insect behaviour is directly reflected in the diversified microbial communities inhabiting the gut (Martinson et al., 2011). The wide narrative of the studies done, particularly with termites and cockroaches, have lay down its effect on the nutritional contributions of gut bacteria in insects living on suboptimal diets (Moran et al., 2012). The gut microbiota

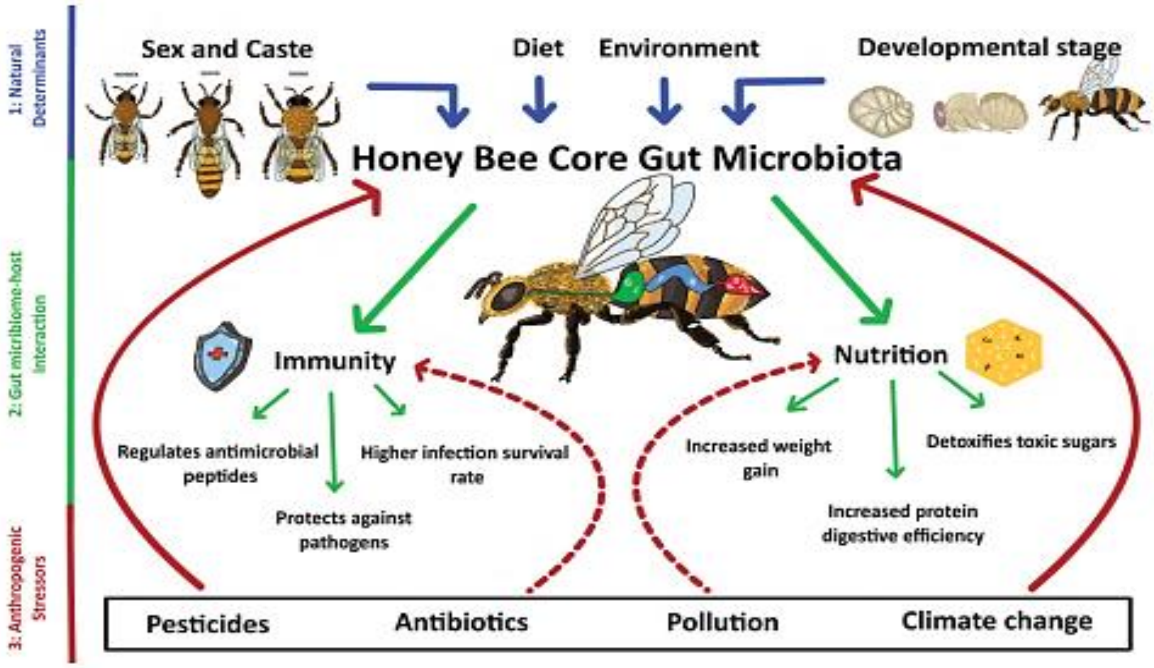


Fig. 1. Honey bee core gut microbiota

represents all the corners of microbial relationships, from pathogenecity to obligate mutualism. The insect gut microbiota has been demonstrated to have profound effect on the digestion of the host, detoxification, developmental processes, pathogen resistance, and physiology. Among the enormous benefits of the microbes present in the gut of the honey bees, the microbes can provide essential amino acids (Nogge, 1981) and vitamins (Douglas, 1998). They also act as the good source of nitrogen and carbon supplements (Benemann, 1973, Dillon & Dillon, 2004) to hosts.

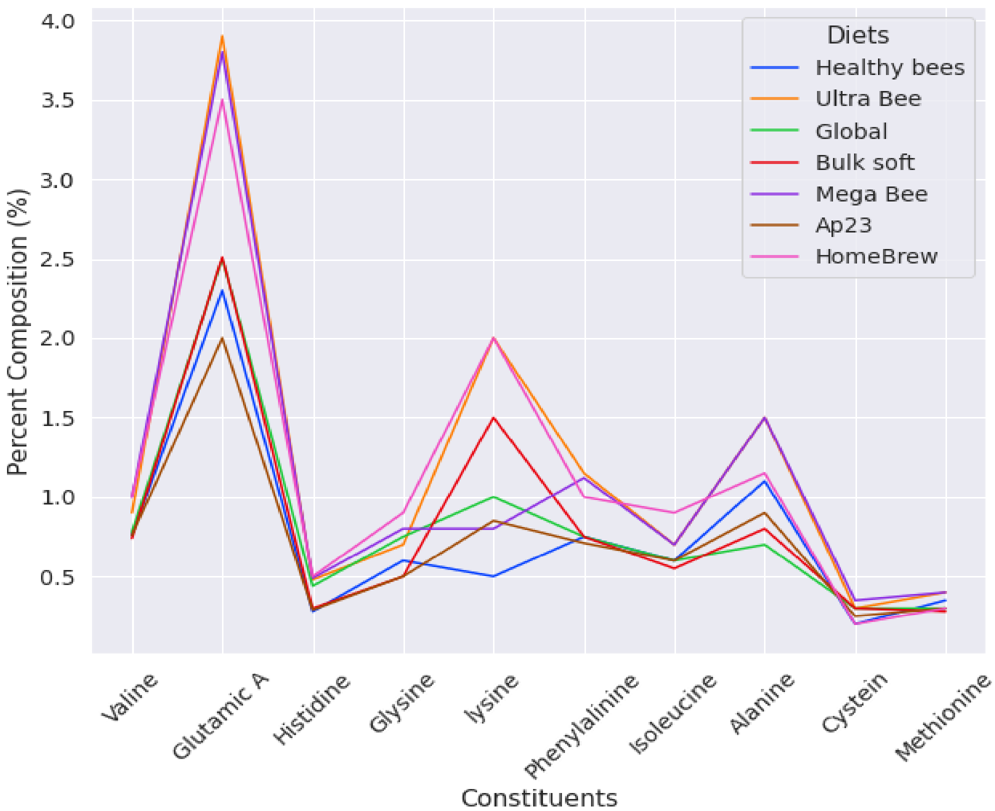
The gut microbes alters greatly the between the different associations such as mutualism and commensalism and in extreme cases parasitism in response to changes in host diet (De Vries et al., 2004). Seeing the significance of the microbial diversity present in the insects gut, the ward off of the resident bacteria population can steeply lessen the flying ability of the honey bees (Ben-Yosef et al., 2008; Dinh et al., 2019). The artificial diet profoundly alters the microbial community diversity and it can get changed also by the honey bees fed with artificial diet (Chandler et al., 2011, Broderick & Lemaitre, 2012, Mason et al., 2014, Yun et al., 2014, Majumder et al., 2019). Moreover, the gut microbiome biodiversity and community structure also get transformed by the diet nutritional composition (protein, carbohydrate and lipids) (Broderick et al., 2004; Ravenscraft et al., 2019; Woruba et al., 2019; Doull 1974). After the way long sequencing of multiple rRNA especially 16S rRNA, it was opined that the predominant occupation of gut lumen was inhabited by some classes Proteobacteria and Bacteroidetes however, the complete analysis of anal pore droplet revealed that the abundance of Proteobacteria was in plentitude (Ricigliano et al., 2017).

6. FUNCTIONS PERFORMED BY BACTERIA

One of the major effect of the insect gut inhabited bacteria is the decreased the disease causing efficiency of the antigen in addition to the nutritional and immune triggering benefits (Raymann & Moran, 2018, Kwong & Moran 2016). Nevertheless the research further revealed the microbial colonies of gram-positive bacteria inside the gut in which predominantly *Lactobacillus* taxa, *Bifdobacterium asteroides* (Anderson & Ricigliano 2017, Kwong et al.,2014, Cilia et al.,2020) and *Snodgrassella alvi* (*Betaproteobacteria*) and *Gilliamella apicola* (Gamma proteobacteria) respectively were highest in number (Kwong & Moran, 2013, Leonard et al.,2020). The depleted health conditions of *Lactobacillus* colonies were studied by non-thriving bacterial colonies (Ribiere et al 2019). On subsequent testing effects of different artifcial diets on honey bee gut microbiota abundance of 16S rRNA of prominent gut taxa were calculated in sequential subdivisions of colonies from each treatment group and at each apiary site. The wide variety of gut bacterial population were deliberated based on nutrition value and physiological functions as the bacterial colonies of *Lactobacillus* spp. and *Bifdobacterium* spp. are interestingly involved in the process of microbial fermentation in an animal microbiota, with not only non-ambiguous utilization of carbohydrate repertoires but undertook the process of sugar toxicant detoxification in pollens (Zheng et al., 2016). Snodgrassella alvi is non-fermentative but participates nutrient-sharing interactions with the fermentative bacterial community (Kesnerova et al., 2017). And as per the diets given in apiary site, most diet effects overrode and no direct relationship were found between gut microbiota abundance and colony size. However, the single protein mainly derived from spirulina has remarkable feature of the bee diet being the reason for ample protein source. The spirulina being the economical microalgae and the source of single cell protein diets are very extraordinary source of feeding in terms of laboratory conditions (Ricigliano et al., 2021). Among the artificial diets the plant based essential oils used as honey bee nutrition supplements they were found to improve attractiveness and spoilage characteristics which was greatly controlled by specific composition of essential oils in the diet, in terms of an antimicrobial effect since essential oils are differential mixtures of compounds and molecules that can put enormous impact on gut bacterial population (Cao et al., 2020, Castelli *et al*., 2020).

7. EFFECT OF THE DIFFERENT DIETARY SUPPLEMENTS

The diversified dietary formulae demonstrated by Ricigliano et al., 2022 (Ultra bee, Global, Bulk Soft, Mega Bee, AP23, and Healthy Bees) extensively across different nations revealed important functional roles in honey bees (Crone & Grozinger 2021, Arien et al., 2020).



Graph 1. Percentage composition of varying amino acids in different artificial diets (Ricigliano et al., 2022)

Table 1. Aminoacid content of different honey bee diets

Composition	Diets						
	Healthy bees	Ultra Bee	Global	Bulk soft	Mega Bee	Ap23	Home Brew
Valine	0.76*	0.9	0.78	0.74	1	0.76	1
Glutamic A	2.3	3.9	2.5	2.51	3.8	2	3.5
Histidine	0.28	0.48	0.44	0.3	0.49	0.29	0.5
Glysine	0.6	0.7	0.75	0.5	0.8	0.5	0.9
Lysine	0.5	2	1	1.5	0.8	0.85	2
Phenylalinine	0.75	1.15	0.75	0.75	1.12	0.71	1
Isoleucine	0.6	0.7	0.6	0.55	0.7	0.6	0.9
Alanine	1.1	1.5	0.7	0.8	1.5	0.9	1.15
Cystein	0.2	0.3	0.3	0.3	0.35	0.25	0.2
Methionine	0.35	0.4	0.3	0.28	0.4	0.3	0.3

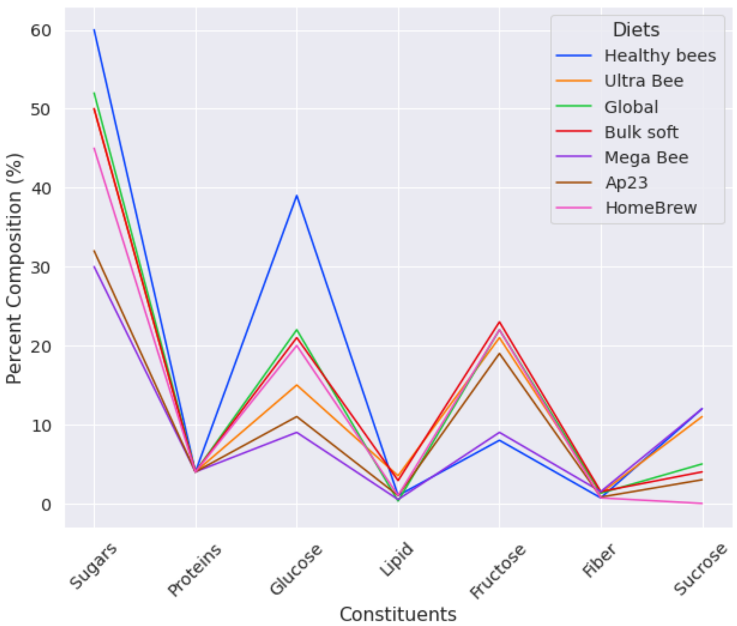
**Signifies percentage composition*

Table 2. Macronutrient content of different honey bee diets

Composition	Diets						
	Healthy bees	Ultra Bee	Global	Bulk soft	Mega Bee	Ap23	Home Brew
Sugars	60*	50	52	50	30	32	45
Proteins	4	4	4	4	4	4	4
Glucose	39	15	22	21	9	11	20
Lipid	1	3.5	0.3	2.9	0.5	1	1
Fructose	8	21	22	23	9	19	22
Fiber	0.7	1.3	1.3	1.5	1.5	0.8	0.7
Sucrose	12	11	5	4	12	3	0

**Signifies percentage composition*

Due to the fact that insects can synthesize varying lipid molecules from carbohydrates (Arrese & Soulages, 2010), dietary lipid requirements for honey bees are not fully comprehended and are confounded by the fact that crude lipid content was highest in the formulation Ultra Bee (3.5%) and Bulk Soft (2.8%) diets and was found lowest in the Global diet (0.2%).



Graph 2. Percentage composition of different dietary constituents in artificial diets (Ricigliano et al., 2022)

Studies conducted by (Ischebeck, 2016) and (Chakrabarti et al., 2019) revealed that pollen supplements contain variety of lipid types and at the cellular levels lipid components are the important constituent of macromolecules such as membrane lipids and lipoproteins (Wegener et al., 2018) where linoleic acid and alpha-linoleic acid are two fatty acids that are immanent for animals (Hulbert et al., 1999) including bees. Nevertheless, it has been elucidated that dietary fiber in a pollen food can modulate microbiota and host animal physiology because the similar crude fiber content of dietary fiber were determined in different pollen diets ranging from 11 to 16 % (Bonvehi & Jorda 1997, Morrison et al., 2020). Keeping in view the hygroscopic nature of the monosaccharide fructose the blend of the sugars is also well accepted by the honey bees. On estimation the total sugar percentage elucidated and the highest level was present in the Healthy Bees diet (60.4%) and lowest in the Mega Bee diet (30.2%). The simple sugars such as glucose and fructose were accounted for the majority of sugars in all diets. After the complete amino acid profiling the following amino acids viz. arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine were are of paramount importance for honey bees due to their indirect wide range of physiological implications. The greatest proportional requirements are for leucine, followed by isoleucine and valine (De Groot, 1953). The pace at which protein synthesis was reached by a given diet formulation, is explicated by the concentration of the essential amino acid (EAA) present in the smallest proportion with respect to the animal's requirements as per Liebig's Law (Crone & Grozinger, 2021). The diet produced the largest number of colonies was Homebrew with an average of 8.1 frames of bees however, the Healthy Bees diet produced the smallest colonies with an average of 2.2 frames of bees. It was further illuminated that the diets low in protein content produced very larger colonies, which points to other factors driving diet effects.

8. PATHOGEN INFLICTING DAMAGE MEASURE

The variable pathogens such as the mite *Varroa destructor*, the microsporidian *Nosema ceranae*, and diferent RNA viruses have direct imprint on the honey bee colony losses. Some of the agents acts as a vector of disease causing pathogens such as viral pathogen vector Varroa mite, helps in the transmission of Deformed Wing Virus (DWV). When the colony population of mites and *N. ceranae* is minimal in amount, DWV generally causes easily detectable asymptomatic infection in most colonies.

9. CONCLUSIONS

The microbial community makes prominent contribution in the gut of the insects where they profusely aid in varying life mediated processes and feeding habits of insect host. The microbial contribution in the gut functioning is of paramount significance relevant from wide perspectives, such as physiological processes, biochemical reactions mediated by enzymes, agriculture, and study of ecology. The symbiotic gut denizens can have huge impact on the vectoring efficiency (Ricci et al., 2012) and developmental time, which indirectly results in the potential disease control. The usage and incorporation of fresh and high-quality material plays very quintessential role in booming and thriving of honey bee larvae before exploiting it for scientific manipulation and strenuous exercise testing of compounds and pathogens. Nevertheless, the larvae reared in natural habitat and contexture conditions will not be distinguishable from the larvae raised on artificial medium. Thus critical and precise examinations are of utmost importance with in vitro-reared larvae. The micro biome in wide diversified insect genera specifically in honey bees plays have been found in playing the quintessential role in regulating the metabolic functions, greatly altered by artificial nutritional diets (Chouaia et al., 2012). There has been limited and very subtle information present regarding the advantages of honey bee nutritional supplements in large-scale field applications. The greater amount of variation has been found in local apiary site environments which majorly depend upon the type of natural pollen material available at the time of testing. The larger colonies have been formed and had led to the enormous growth of the colonies when artificial man made diet is in richest amounts of proteinacious content. As far as present study is concerned, the macronutrients solemnly could not be attributed to diet effects. The population and viability of the colonies is not only affected but feeding the colonies with artificial diet supplements paved a great potential impact on multiple processes such as metamorphoses, leading to the lower emergence rate in honeybees (Vandenberg & Shimanuki, 1987) however, the rate of metamorphosis got better when feeding was done with ample proportion of uniform diet to metamorphose as compared to varying concentration of diet given. The colony size and average bee weight were taken as a strong tool to validate the dietary essential amino acid deficiencies relative to leucine. Efficiency of diet in bringing the particular changes is directly correlated with the nature of the diets in particular with the nutrient concentration rather than their origin. The aim of this review confines the commercial diet formulas and demonstrate that majority of the dietary formulations have supplied some overlooked nutritional factor such as essential micronutrients. In the context of our study some completely artifcial diets proved to be helpful in terms of effect of pollen nutrition. In a concluding remark it was manifolds exploited that ready made feed can enhance the health benefits and performance of commercially managed bee colonies. The aftermath conclusions obtained from the studies carried by many authors unveiled the diagnostic studies which are necessary for comprehension of nutritional requirements and the essence of supplemental feeding on honey bee colonies. On the quest of advancement of bee diets it will lead to potential improvement in the supplement freight and enhancement of pollination vitality by fostering larger and significant colony expansion. In the developed European nations, during the dearth phase of the bee flora the feeding regime has catered many specific beekeeping operations also.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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