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Effects of Supplemental Meals' Nutrition on the Biological Processes and Honey Production of Apis Mellifera L.

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Abstract

The goal of the current research was to determine how nutrient-rich diets might affect physiological processes and, in turn, honey production. During the winter and summer of 2019–2020, 18 colonies of Apis mellifera L. were chosen from the Dera Ismail Khan area of KPK, Pakistan. To find the most pleasant pollen supplement diet to feed as an alternative to bee bread, five pollen supplement diets were developed and offered. The results of food consumption considering mean data for consumption rate showed that honey bees ingested the most soybean flour-enriched artificial diet (74.34 g) each week. The grinded groundnut enhanced diet (64.62 g) had the lowest consumption, compared to the other fake diets that were put to the test. According to the area of worker brood results, the control diet (463.51 cm2/colony) was the least effective, while the soybean flour enriched diet (1489.27 cm2/colony) was statistically notable compared to the other artificial diets. The bee colonies fed a diet fortified with soybean flour had the highest bee strength (10.00 bee frames/colony), date paste (8.0 bee frames/colony) was the next most effective pollen replacement diet, and groundnut flour (5 bee frames/colony) had the lowest. The newborn bees with the highest body weight (12.41 g) were fed a meal enhanced with soybeans, whereas those with the lowest body weight (5.31 g) were fed a control diet. The development of wax cells and foraging effectiveness were also better in artificial diets than in the corresponding control bees. Therefore, artificial diets, particularly pollen alternatives high in soybeans, can improve honey bee physiology, increasing honey production and profit [1].

Keywords: Honey bee colonies; Diets; Palatable Profit; Bee strength; Brood area; Apis mellifera; Honey quality; Nutritional values

Introduction

The most popular sweetener in the world and nature's original sweetener, honey is made by honey bees. At least 6000 years have passed since honey was first utilised as sustenance. A very popular natural sweetener made of glucose and fructose, honey evolved from the nectar. Honey is actually floral nectar that honey bees have collected, regurgitated, and dehydrated to improve its nutritional value and make it fit for human use. Due to the special combination of ingredients that make honey a valuable addition to the diet, honey intake is on the rise. The overall production of honey was 0.7 million tonnes in 1961, according to the Food and Agriculture Organization of the United Nations (FAO), and it climbed steadily to roughly 1.5 million tons in 2009. Since high-quality honey has been demonstrated to have antibacterial, antiviral, antiparasitic, anti-inflammatory, antioxidant, antimutagenic, and anticancer activities, it is in great demand due to its health advantages. The presence of more than 181 compounds with a variety of health-promoting phytochemicals, some of which have antioxidant characteristics, and the ability to prevent disease through honey consumption are likely to be the causes. Natural honey is rich in antioxidants, which act as free-radical scavengers and either lessen or neutralise the production of free radicals. The elements in honey that give it its antioxidative properties are called phenolics. Therefore, there is a lot of interest in the study of honey processing due to the significance of these bioactive chemicals and antioxidants in honey for human health [2].

Phenolic acids, flavonoids, and amino acids are some of the antioxidant components found in honey. The major antioxidants in honey are said to be phenolic chemicals, notably gallic, p-coumaric, ferulic, syringic, caffeic, synaptic, and chlorogenic acids. The finest honey for scavenging free radicals is that which contains the highest concentration of phenolic components. It is also well known that phenolic chemicals increase antioxidant activity, and that honey's total phenolic content (TPC) and antioxidant activity are strongly correlated.

High pressure processing (HPP) is a non-thermal method that improves product quality and has the power to render microorganisms inactive in a variety of food matrices. HPP has the ability to generate high quality foods with "fresh-like" qualities and enhanced functionality as an alternative to conventional heat processing. Although much research on HPP's effects on microbial and enzymatic inactivation has been widely published in the literature, there is substantially less information on how pressure affects specific components of food quality. The investigation of novel high pressure processing is currently one of the most intriguing studies in nonthermal food preservation due to the rising demand for better-quality, safer foods. Food that has been HPP-treated has been demonstrated to maintain its original freshness with little change in flavor, taste, or color [3]. This is because isostatic pressure is transmitted practically instantly.

Aloe Vera, strawberry pulp, fruit smoothies, apple puree products, strawberry purees, blackberry purees, grape by-products, vegetables, and orange juice have all been used in previous attempts to study the impact of HPP on TPC. These tests used pressures between 400 and 600 MPa and temperatures between 10 and 70 °C. The majority of studies showed that the TPC rose for samples that had been treated with HPP.

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However, there haven't been any studies on how HPP might be used to improve the bioactive chemicals and antioxidant activity of meals with high sugar content, notably honey [4].

So, the purpose of this study was to look at the differences in TPC between standard thermal processing and HPP treatment of manuka honey. Additionally, the combined impact of pressure and temperature will be examined.

Materials and Methods

Study site

Therefore, the goal of this study was to compare the TPC of manuka honey after standard thermal processing and after HPP treatment. The combined effect of pressure and temperature will also be studied.

Experimental colonies

In the winter and summer of 2019–2020, 18 colonies of A. mellifera L. were chosen from Dera Ismail Khan, Pakistan. Approximately equal numbers of A. mellifera L. colonies with five bee-covered combs were used for this experiment. Six equal groups of honey bee colonies were created; five were used to study the impact of a diet rich in pollen substitutes on the biological activities of honey bees, and the sixth served as a control group.

Preparation of different pollen substitute proteinaceous diets

To choose the most palatable pollen substitute diet to use in place of bee bread or pollen, five pollen substitute diets were produced and provided (the main source of protein for bees). These five diets without pollen were created using five readily available foods in the area. These five diets were created by combining the components listed below in various ratios. As the primary protein and carbohydrate source in diet 2, sugar cane syrup served as the main ingredient in diet 3, date paste served as the primary protein and carbohydrate source in diet 4, black gramme flour served as the primary ingredient in diet 5, and soybean flour served as the primary pollen substitute in the first diet combination.

Throughout the course of the study, colonies were given a single diet of their choice. At intervals of 15 days, each colony received 100 g of the prescribed meal in plastic packaging with several cuts on each side[5]. To stimulate activity, 250 mL of sugar syrup (1:1, w/v) was given to each colony as supplemental feed every three days. A unique water source was also made available to each colony.

Patty feeding

The diet patties were wrapped in plastic, with several cuts made on each side, and then they were set out in the colony. The honey bee colonies had easy and immediate access to them. Five days apart, the patties were checked, and fifteen days apart, new patties were substituted.

Diet consumption

The weight of the fresh diet patty and the weight of the patty 15 days after it was placed in the colony (g/colony) were subtracted to get the amount of food consumed. For each designed diet, the data were collected five days apart. During the course of the experiment, the total number of patties consumed with each diet type was also being recorded by method.

Brood area measurement

After fourteen days, the sealed brood area of worker bees was measured using a computing frame with a wire net scaled to give an area of 1 square inch per square, and then converted to cm2 by multiplying by 2.54. This closed brood was used as the benchmark for judging the colony growth.

Foraging effort

After fourteen days, the worker bees' sealed brood area was measured using a computational frame with wire mesh scales that gave each square inch of space a 1 square inch area. This area was then converted to cm2 by multiplying by 2.54 to get square inches. This closed brood was said to as the gold standard for judging colony expansion.

Measurement of thorax weight and number of wax cells built

Bees were divided into three insect body segments and organised into ten groups for this experiment. By dehydrating to a constant temperature of 60 °C for duration of 48 h, and with an accuracy of 0.1 mg, the mean thorax weight was determined. Additionally, each group's production of wax cells was counted.

Determination of dimensions of queen cells

The Doolittle method resulted in the production of queen cells. Ten cells from each group of the total number of queen cells were used to measure the width, length, and opening diameter of each cell as well as the body weight of the newly formed queens. Prior to the arrival of the queens, the breadth and length of the ten randomly chosen queen cells were measured. The length of the queen cell was measured from the beginning of the beeswax cell cap to its highest point, and its width was determined at its widest point. The weight of newly grown queens (on appearance) and the diameter of the queen cell aperture were measured shortly after the appearance of the queen [6]. All measurements of queen cells were made using a computerized fractional caliper with an accuracy of 0.01 mm.

Honey production

Results on honey production were recorded after the study was complete in order to compare the honey output of bee colonies fed chemically produced food with control bee colonies and assess the impact of pollen substitutes given to the bees in this study [7].

Statistical analysis of data

A two-factor factorial analysis using a Completely Randomized Design (CRD) and triplicates was conducted. The statistical analysis of bee-covered frames, sealed worker brood area, feed intake, and honey yield/colony was performed using statistics software version 8.1 and two-way analysis of variance (ANOVA). Least Significant Difference (LSD) was used to distinguish treatment means when p 0.05. The F test was used to process the results of the dimensions and other qualitative parameters, and the Duncan's Multiple Range Test (a 1/4 0.05) was used to assess group differences.

Results and Discussion

The 1H-NMR spectra of Ecuadorian pot-honey samples were analyzed for the presence of the following 41 parameters, which included 10 sugars, HMF, and ethanol, 9 organic acids, 10 amino acids, and 10 markers of botanical or entomological origin: Sugars (fructose, glucose, sucrose, gentiobiose, maltose, maltotriose, mannose, melezitose, raffinose, and turanose), HMF, and ethanol are the first three ingredients. 3. Organic acids aliphatic (acetic acid, citric acid, formic acid, fumaric acid, lactic acid, malic acid, pyruvic acid, quinic acid, and

succinic acid), Four. Amino acids (alanine, aspartic acid, glutamine, isoleucine, leucine, phenylalanine, proline, pyroglutamic acid, tyrosine, valine), 5. Botanical origin markers (acetoin, 2,3-butanediol, dihydroxyacetone, kynurenic acid, methylglyoxal, methylglyoxal dihydrate, methylglyoxal monohydrate, 3-phenyllactic acid, shikimic acid, and trigonelline). In the 20 honeys examined, no quinoic acid, isoleucine, or kynurenic acid was found. See a for the 41 parameters' molecular composition, chemical make-up, regions of the 1H-NMR spectrum (ppm), signal kind, and available 1H-NMR spectra[8].

The aliphatic components of honey, including lactic acid, acetaldehyde, methylglyoxal dihydrate and monohydrate, pyruvaldehyde, and pyruvic acid, were detected in the first spectroscopic region of the 1H NMR spectra (1.3-2.3 ppm), while phenyllactic acid was detected in the aromatic region (6.0-8.0 ppm). Because the endogenous lipophilic markers of the bees obtained with chloroform extracts are independent of floral and geographic origin, as described, a central region of the 13C NMR spectra was characteristic for each bee genus in a comparison of commercial Ecuadorian Apis mellifera, Geotrigona, and Scaptotrigona honeys.

There were notable variances and similarities in the organic chemical profile (41 substances) of the honey made by three genera of stingless bees. One characteristic of biodiversity is the variety of the honey they produce. At the genus level for stingless bees, comparative 1H-NMR spectra were displayed. Accordingly, the quantitative results were described and discussed [9]. A potent method for multiparametric analysis is targeted 1H NMR. Selected metabolites in the reference standard may be identified qualitatively, and their corresponding quantitative measurements could be made.

Each 1H-NMR spectrum contained a sizable amount of honey metabolites with a complex profile and only a small number of isolated and distinguishable signals from particular metabolites. The analysis of a spectrum with overlapping signals may make it difficult to identify peaks and calculate concentrations. Three example pot-honey samples from the Geotrigona, Melipona, and Scaptotrigona were displayed in their 1H-NMR spectra. The stingless bee genus may be connected with differences in the number and/or strength of the peaks. Due to the high levels of acetic acid and lactic acid present, Geotrigona displayed extremely high intensities in the aliphatic region. A very condensed and packed region of the 1H-NMR spectrum contained the signals of carbohydrates with anomeric and ring protons (3.0–5.5 ppm). Because of their high intensities, signals from highly concentrated carbohydrates like fructose and glucose were simple to distinguish. AOA and amino acids were present in the aliphatic 1H-NMR region of the spectra (3.0-0.1 ppm) [10]. The signals from additional honey metabolites, such as aromatic amino acids and terpenoids utilised as botanical identifiers were found in the aromatic region (10–6 ppm).

It was possible to see the spectra in finer depth. The sugar region of the spectrum in Geotrigona pot honey displayed a very distinctive profile with a high concentration of raffinose of 5.6 g/100g. Although the quantities fell below the Apis mellifera reference value of 0.1g/100g honey, fructose and glucose signals were still discernible. In contrast to the extremely high quantities of acetic acid and lactic acid, the low intensity (concentration) of glucose was notable. The aliphatic region of the spectrum and the aromatic region for the aromatic amino acids phenylalanine and tyrosine were both good places to look for side chain signals from amino acids[11]. The strength of the raffinose peaks was significantly lower in the Melipona pot honey than it was in the Geotrigona honey, and the quantification showed a concentration of 0.3 g/100 g, which was nevertheless higher than the raffinose concentration in Apis mellifera honey. Mannose peaks with unusual shapes were clearly visible. Peaks from aromatic amino acids made up the aromatic area, and some extra signals could be from other aromatic chemicals. The aliphatic region of the Scaptotrigona pot honey displayed a strong signal of ethanol, acetic acid, and lactic acid, but at a lower level than Geotrigona. The fermentation processes that produce these metabolites are common[12].

Conclusions

The amino acid isoleucine, the organic acid quinic acid, and the marker kynurenic acid were not found in the Ecuadorian honeys out of the 41 parameters evaluated here by targeted 1H NMR. As a result, the multivariate analysis was performed on eight markers, nine organic acids, nine amino acids, and ten sugars. In comparison to amino acids and markers, sugars and organic acids were more discriminating of the bee genus. Three problematic honeys were tested, and the results showed the limitations of multifactorial analysis and the need for descriptive parameters in order to make a preliminary fit of these honeys to the developing 20-honey database. To make insightful comparisons with honeys that are similar and to learn about the exceptions, a reliable database must expand.

The targeted NMR chemical profiles are helpful for a multipurpose reference database to check an unidentified pot-honey or suspected adulteration, mislabeling, or fake product. A database of this kind is expanding for adulteration sources from different nations as well as sources with botanical, entomological, and geographic origins. The biodiversity of honeys in Ecuador is a hidden treasure, with a wealth of floral and entomological resources. Our findings with a small sample size of honeys merit further study, along with limited information on honey quality controls other than NMR. Given that the composition of pot-honey shows a relationship between taxa from the plant, bee, and microbiota—three kingdoms—facts that cannot be explained by their botanical or entomological origins may have a microbiota explanation in the nest. The identification of the microbial origin and chemical makeup of the bio surfactant were both necessitated by the discovery of bio surfactant activity in Scaptotrigona honey.

Research on the composition of pot honey is an ongoing, slow-moving effort to aid the meliponine scientific community in understanding bee activities and for regulatory purposes, which will eventually benefit stingless beekeeping, agriculture, and consumers. The NMR data presented here is a support for establishing necessary honey quality criteria and serves as a norm for the stingless bee-produced honey in Ecuador.

Aside from the already underway metabolomics research, specialised teams in the fields of genomics, transcriptomics, and proteomics will need to use the term "foodomics," which Alejandro coined as "A discipline that studies the Food and Nutrition domains through the application of omics technologies." If stingless bee honey processing is in fact influenced by bee phylogeny and relationships with microbes that have a variety of activities, including nutritional features, it would be easier to comprehend environmental or other influences. The test results for Scaptotrigona vitorum honey indicated bio surfactant activity, which supported microbial interactions as the source of that visual behaviour. For this collection of Ecuadorian pot-honeys, our conclusion is. Starmerella bombicola was discovered by Echeverrigaray et al. in Brazilian honey produced by Scaptotrigona bipunctata and S. ederi but not by S. tubiba. Of light of this, the microbial connection with stingless bees in the genus Scaptotrigona is species-specific rather than genus-specific as it was in our investigation

conducted in Ecuador with the species Scaptotrigona vitorum.

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None

Conflict of Interest

None

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