



EFFECTS OF BEES FEEDING ON SOFT DRINK ON BIOCHEMICAL COMPOSITION OF HONEY SAMPLES AND PROBABILITY OF ITS IMPACT ON BEE HEALTH AND VITALITY

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ABSTRACT

In this study, the factors that had an impact on the quality of honey produced in southern Saudi Arabia were examined. Honeybee colonies are supported by two kinds of colonies. The nutritional requirements of honeybee workers can be classified into two groups: 1) colony nutrition using Pepsi and pollen supplementation, 2) other nutrition, determined by the natural gathering of nectar. The bees being fed on Pepsi were observed to be strongly influenced by deficiencies of essential nutrients. There was a decrease in the number of bees. The conditions of supposed malnutrition also had an impact on the quality of developing workers because of the lack of essential nutrients in Pepsi, causing high rates of mortality. Therefore, biochemical constituents of two honey samples acquired from different kinds of honey were analysed to determine their qualities. Statistical results for the total of glucose and fructose, glucose, fructose, Hydroxy Furfural Diastase (HMF) and disease were obtained as (72.98±0.05, 61.36±0.94), (34.28±0.10, 28.64±0.54), (39.00±0.44, 31.60±0.35), (58.06±0.70, 49.38±0.59), (24.14±0.46, 20.18±0.48) respectively. Moisture and sucrose (0.89±0.01, 2.60±0.15) (72.98±0.05, 61.36±0.94) were found to a greater extent in sample B compared to sample A, respectively. There were statistical variations in the content of honeys in the two kinds of feeding bees, where $P < 0.0001$. Natural honey contains various groups of substances which offer it its unique characteristics and make it one of the most extensively sought products. Honey has nutritional, medicinal, as well as industrial properties and is a vital product in the international market as it functions as a foreign exchange earner for various countries. Honey production (bee-keeping) is capable of developing as a significant arboriculture and forest-based industry that can be a major source of foreign exchange, provided the international standards are fulfilled. The kind of nutrition foraged by the bees determines the exact chemical constitution and physical features of natural honeys. The different properties of honey are also affected by the variations in climatic conditions and vegetation. The climatic conditions in south of Saudi Arabia are arid, semi-arid and humid, with different agricultural activities and production of different kinds of vegetation that can have an impact on the natural composition and properties of honey in different seasons, excluding extreme climatic conditions. It is suggested by the findings of this study that the samples are comparable to samples from different areas of the world and fulfil global standards.

KEYWORDS: Pepsi, nutrition, glucose, sucrose, fructose, soft drink, insulin, bee's insulin, Hydroxymethylfurfural, Honey diastase, insects' hormones, rats, body weight, pancreatitis, blood glucose.

The impact of feeding rats on the two kinds of honey was recorded in the applied study. The obesity of rats in group (A) was compared to that of rats in group (B) as well as the control group, with a considerable increase in the quantity of glucose and insulin within the blood, which is typically associated with symptoms of diabetes.

There is not a considerable difference between the action of such important systems like protein synthesis, muscle contraction and cell metabolism in humans and insects.

Most of the hormones separate from vertebrates are also found in insects. The rate of glucose being transmitted through cell membranes is regulated by insulin, which also provides nutritional information that is utilized by organisms to manage their development. The function of insulin to manage growth has been conserved to a large extent in insects.

Laboratory results of this study demonstrated weakness and high mortality of beehives fed on Pepsi compared to those colonies that feed on natural nectar. These findings suggest that there is a large probability of disorders in

metabolic processes, hormones associated with digestion, energy generation and absorption.

Study Aims: There are three particular objectives of this study, the first of which is to generate Saudi honey of high quality that fulfils the global standards of quality and trade requirements. The second objective is to obtain the support of Saudi research organisations to perform studies on the physiological fluids and hormones on bees that have been illegally fed on soft drinks by the bee-keeper in certain Saudi farms. In addition, it seeks to analyse and assess the effect of this on bee vitality, hormonal problems and reproductive and productive abilities, which is directly associated with the economic aim.

The third aim is to demonstrate the impact of commercial frauds carried out by a few bee-keeper while producing honey in non-season by feeding them soft-drinks, and its impact on the health of consumers.

The final aim is to rectify certain conventional misconceptions prevailing in conventional Saudi communities regarding honey therapy.

INTRODUCTION

Natural honey includes various groups of substances which offer it its distinct nutritional and medicinal properties, making it one of the most extensively sought products. According to Codex Alimentarium Commission, honey was described as the natural sweet substance created by the honey bees, *Apis mellifera*, from the plants' nectar (blossoms) or from the substances secreted by the living parts of plants or substances excreted by plants sucking insects on the living parts of plants which is collected by honey bees, altered by mixing it with particular substances of their own, deposited, dehydrated, preserved and kept in the honey comb so that it becomes ripe and mature.^[43,44] It is believed that the bees create honey to serve as a source of food for themselves during times of scarcity or when the weather is quite rough.^[80]

Natural honey is, in essence, a sticky and viscous solution, comprising of 80–85% carbohydrate (fundamentally glucose and fructose), 15–17% water, 0.1–0.4% protein, 0.2% ash and very small amounts of amino acids, vitamins and enzymes, in addition to other constituents such as phenolic antioxidants.^[80] It is believed that each of these minor substances possess distinct nutritional or medicinal qualities and this unique mixture is responsible for providing a variety of uses of natural honeys.^[80] All honey samples essentially have the same main constituents; however, the actual chemical constitution and physical characteristics of natural honeys are different in terms of the sources of nectar that have been foraged by the bees.^[80,37,50] In addition, the different qualities of honey can also be influenced by the variations in climatic conditions and vegetation.

Nectar and honey: Glands present at the base of the flowers, called nectarines, secrete nectar. Nectar is obtained by field bees from blossom in the field. The sucrose sugar is excessively present in the nectar in this stage, along with a little laevulose (fructose) and dextrose (glucose) as well as a high moisture content. Traces of other substances are also present, for example vitamins, minerals, aromatic substances, pigments, nitrogen compounds and organic acids. Various steps are involved in the conversion of this nectar into honey by the bees. The nectar collected in the beginning is kept in the honey sac of the returning field bee. There is addition of an enzyme known as invertase to the nectar when it is in the honey sac of the bee. The nectar, which is mainly a sucrose solution, is transformed by invertase into a solution that is essentially laevulose and dextrose. The ripening nectar is then preserved in the beeswax cells where there is a decrease in the moisture content to 13–18% by manipulating and fanning the house bees. The bees create a cap of beeswax when the honey has ripened.

There are medicinal, nutritional and industrial uses of honey, which is a significant commodity in the global market, serving as a source of foreign exchange in various countries. Bee-keeping has been carried out for a long time in the southern part of Saudi Arabia. It has been a component of typical agricultural organisation in certain societies in the country, while in various parts of the world, honey production mainly takes place at a subsistence level.^[35-101] Nonetheless in southern Saudi Arabia, there are extensive quantities of honey in beehives, and it has been accepted that honey production (bee-keeping) is capable of turning into a major component of agro horticultural and forest-based industry, which may turn into a significant source of foreign exchange provided international conditions are fulfilled. A varied combination of plant and animal life is present in the forest ecology in southern Saudi Arabia. There is moderate climate in this part of the country. The temperature does not go over 30° C all through the year, winter temperatures are low, going down to 5 ° C, and at times even less than 0° C. In addition, around 500 mm of rainfall takes place annually, which is the highest in the country which is influenced by the winter and seasonal rainy season because of its position on the equator's side. The winds are south-westerly to westerly, and north-westerly dry in the spring, and agricultural activities and blossoms are different for distinct kinds of vegetation, which can have an impact on the natural constitution and qualities of honey in the region, because of which it is quite appropriate for apicultural activities.

The number of workers in Honeybee (*Apis mellifera* L) colonies are quite large, ranging from 15,000 to 60,000 bees.^[125] A large quantity of food is needed by these large number of bees to ensure their survival and growth. When there is ample nutrition, the development of healthy bee colonies is ensured.^[31,113,114]

Honeybee colonies require nectar, essentially as a source of carbohydrate, in extensive amounts. Nectar is required by the honeybee workers as an energy source, particular during the winter months when they are seeking to maintain the brood temperature within a range of 33 to 36 °C for the survival of the colonies.^[121] They do this by warming the brood caps superficially, or internally by heating the bees.^[86,123,25,126] stated that in the winter months, there was high decrease in colony weight, suggesting that in this critical time of the year, there was high consumption of honey. It is believed that the low temperatures during winters has led to losses of honeybee colonies in Saudi Arabia.^[7] In areas of the USA where the mean temperatures are low, there have been extensive colony losses.^[34] Usually, these colony losses take place during the winter months.^[99] It has been estimated that these losses amounted to 19% in Belgium in 2009/08 and 22.5% in the USA in 2011-12.^[126] It has been reported that starvation was one reason for colony losses from 6.0 to 17.8% in the winters in Austria.^[32] It was found that winter colony losses in Germany ranged from 3.8% to 15.2% in 2004/05 and 2005/06.^[66] The deficiency of nectar sources and inappropriate conditions for foraging in the winter months adversely effects bee colonies. Therefore, it is important for beekeepers to offer alternatives to nectar to the colonies. A widely known fact is that pure sucrose is the ideal alternative to nectar.^[19] however, it is important to look for more cost-efficient alternatives that have sufficient nutritional value for honeybees. There are certain feeding sources like heated or old honey that are not suitable for bees, whereas acid-hydrolysed carbohydrates may be deadly to bees.^[36] No benefits of feeding high fructose corn syrup or honey in place of sucrose syrup were presented. When fed to bees, grape syrup decreased survival rates and led to dysentery.^[20] There was greater brood development in colonies fed on honey in field conditions.^[13] The issues inherent in liquid honey are that it is capable of spreading disease within colonies and has high viscosity. When winter bees are provided an appropriate source of energy, they are better able to survive in cold climates. This study aims to examine the various feeding alternatives to make the bees more capable of surviving in cold weather. Two types of feeding alternatives were examined; natural nectar and soft drink (Pepsi). The impact of feeding alternatives of the quality of honey was examined by performing a laboratory analysis of its constituents, as well as its effect on consumer health, particularly those patients who were well known for being treated with honey for different illnesses, for example diabetes. A significant management tool for beekeepers is providing white sugar (sucrose) to honeybee colonies. Its purpose is to serve as a supplement when stored honey is short so as to avoid the colony from starving, or to entice a colony to promote breeding artificially. A valuable part may also be played by feeding sugar syrup in causing a greater number of field bees to forage for pollen from the hive. This will make them more critical in pollinating various economic crops. There are various distinct ways in which sugar can

be fed, where each method has certain advantages and drawbacks. It is preferred to feed while sugar (sucrose) to the bees. Various other products are capable of including substances that may not be beneficial for the health of honeybees. When bee colonies have access to a natural flow of nectar, they should not be fed sugar. When bees are left on their own, they obtain nectar from flowering plants during season and preserve this nectar in the form of honey. The process of obtaining nectar and ripening it to create honey involves a chemical process whereby sucrose (nectar) is converted into fructose and glucose, which are the key sugars in honey. This is attained through enzyme activity that takes place naturally in the flower nectar and also when included by the bees. When nectar is easily available, brood rearing occurs and the colony population increases. A stimulating impact on the colony is not created when just the stored honey is available. Instead, a conservative attitude is shown by the colony by restricting brood rearing and colony expansion. A colony of bees acts in this way so as to increase the survival chances of the colony within a natural system. Access is needed by a colony of bees to stored honey or the nectar for survival. When both of these are absent, the colony will die in a few days because of starvation. A temperature of 20°C will be maintained by a cluster of bees without brood, while the temperature will be 34-35°C when a colony is taking care of young brood. There have been considerable increments in the requirements of colonies for greater volumes of honey/nectar. It is quite normal for colonies to starve in early spring because they have consumed all of the stored honey and fresh nectar is absent. The reason for the absence in nectar may be the extreme weather conditions that forbid the flight of bees. When honeybee colonies are actively handled by providing sugar, the survival rate of the colonies will increase. When sugar is fed strategically, it becomes possible to cause artificially breeding of a colony, which increases its population while expecting that a significant flowering event will occur in the future, offering an additional amount of nectar. When syrup is fed, a greater problem can be posed by ants. Spills should be avoided and any issues that may occur when ants come into the hive to collect the sugar syrup should be kept in check.

Bee breeding is characterized as the involvement of the gardener in the task of supplying bees and nests with the useful food that helps to generate a decent quantity of honey, this method is accomplished by offering some of the natural substances such as: Honey consumption is intended by honey to pay for and deliver those trees. The method also intends at stimulating the breeding of youthful bees and providing them with a total health and nutrition atmosphere, but in the scenario of absence of blossoms and the placement of nectar such as late Saudi winter or summer that is very hot, the beekeeper need to provide the required nutrition to bee colonies so that they can start producing honey.

In the north of the nation, beekeepers carry bees on beverages such as Pepsi beverage in the lack of real forms of nutrition owing to severe weather circumstances to keep consistency of manufacturing and financial gain. A beverage recognized to be detrimental to hygiene for its synthetic recipe.^[110,29] The U.S. Congress in 1906.^[61] had issued a law that bringing maize sugar to honey is a business deception. Legislation in the manufacturing of honey is one of kind in the entire world.

In fizzy drinks with added sugar, Pepsi item is usually available. Around the globe, in advanced nations, Coca Cola and Pepsi have also been liable to use artificial sweeteners that produces many types of illnesses.^[79,4,24,48,74,19, 99,127] Kids must be completely limited from taking aspartame goods. In addition, nicotine and sugar are very habit forming, contributing to other illnesses such as diabetes, kidney problems, hypocalcemia in kids Metabolism Level reduces, obesity is the primary cause of illnesses affecting the body, lungs and kidneys.^[128] Scientists also claim that a massive gain in weight can lead to the development of cancer cells within the body (Striegel-Moore, 2006;).

Likewise, diabetics should never contact drinks such as Coke or Pepsi because it doubles the blood sugar level. These beverages should be avoided by non-diabetic individuals to prevent cancer.^[133,92] A near connection among fizzy drink and decomposition of the tooth. The intrinsic oils and sugar content have both acid and cariogenic ability, leading to tooth decay and future decay of the enamel. Coke or Pepsi's pH level is 3.2 which is very large. This pH level determines a liquid's acidic essence. These drinks are therefore toxic in origin and can very rapidly destroy teeth and enamels.^[110]

Analysis gives evidence that that Coke or Pepsi bottles are covered with such chemicals that with frequent intake can cause reproductive issues.^[72] And a lifetime practice of causing caffeine in the body.^[17] Honey cheats are a very delicate topic and have distinct ideas depending on the distinct areas, there are many ways of cheating honey with distinct techniques and increasing day-to-day avoidance, which is often a concern for customers, especially for famous organizations with certain chronic diseases such as diabetes. Some honey retailers are attempting to challenge the others, they have proposed some basic exams that they distribute among customers in order to persuade them of the excellent outcomes they generate for the honey, but they are all incorrect trials. Although publicly marketed, the kinds of adulterated honey drop into the study of requirements and norms, but are personally sold. In the regionally manufactured honey experiment, implementing worldwide standards of quality render it the only authorized route to distribute and encourage honey.

Physicochemical judgments were made using the techniques of the European Honey Commission.^[27] In a 10g/75ml tea blend in demineralized water, pH and

dielectric constant were defined. In the same medium used for pH assessment, free, lactic and complete acidity were titrated.^[4] Optical index and comparison with Chataway graphs were used to determine the water quality.^[107,106,105] As per Cough.^[46] the proline quality of honey has been calculated. Hydroxymethylfurfural (HMF) concentration was determined using Winkler's technique.^[36] As per the operation of Schade et al.^[119] and Siegenthaler.^[124] the diastase and invertase function of honey extracts was ascertained. For scientific data analysis, ANOVA,^[52] was conducted. Chemical determinations for HMF and Diastase action were made in this research.

Hydroxymethylfurfural (HMF) is an endocrine disruptor created by some glucose being dehydrated. HMF is virtually present in new meat, but it is obviously produced during heat processes such as washing or baking in meat comprising sugar. Among many other drugs associated with taste and colour, discovered in fizzy drinks such as Pepsi. In both the Maillard reaction and during caramelization, HMF is created. It is rapidly produced in these ingredients during processing. (HMF) is a honeybee poisonous product.^[31] Longer burning of honey increases this material.^[94,115]

The activation of honey diastase (DN) is a quality factor influenced by honey retention and cooking and is therefore an indication of honey sweetness and excessive heat. While there is a great normal variety in diastase, it has proved helpful to use the norm of a lowest DN rating of 8. More than 92% of the natural fruit specimens (c. 20 000) and therefore more than 88% of the sales rice specimens (c. 1000) had a DN higher than 8 in over a longer period of time, regular water command at the IHA.^[115]

Vertebrate-type hormones in insects: Insects had once been thought to be free of genes and even absence intelligence, and it is still shocking to know that so many vertebrate life procedures can be discovered in insects as well. Since animals are the older community, it is likely more accurate to relate to the hormones of the insect sort currently in vertebrates. There is no significant difference between us and insects in the activity of such vital processes as protein synthesis, body contraction and tissue metabolism. The instance of the woman rabbit flea, which relies on the hormones flowing in the blood of its pregnant woman vertebrate victim to replicate, finest describes an idea of the connection that remains among insects and vertebrates. Thus, this should not arrive as a wonder that many genes earlier separated from vertebrates are also available in insects, although their simultaneous roles have yet to be completely determined, and significant variations in the composition of amino acids challenge their genuine homologies with vertebrates. Insulin regulates the frequency of transportation of glucose throughout cell membranes and transmits nutritional information that bacteria use to

regulate their development. Its development regulation features have mainly been preserved in invertebrates.^[87]

Materials and Methods: Throughout the winter and spring seasons (November 2017 – April 2018), trials were performed on a barn in the town area near AL-Baha, in the southern part of Saudi Arabia.

This research was conducted in several areas of the Arabian Peninsula on the native plant species *Apis mellifera jemenitica*, one of the purple bee communities. Characterized by adjustment and disease resistance in Saudi Arabia under the prevalent economic circumstances.^[6,8] Which Ruttner^[14] defined as tiny, brief-tongued insects. Comparatively short are the legs and wings.

The woman is distinguished by a comparatively big volume, a brown and yellow fusiform stomach. The men appear to be dark brown compared to the woman employee, white to gray in colour, dotted with flowers. The bee employee abdomen has golden flowers, brief leaves, and the median duration of the tongue between 5.5-4.4 mm.^[49]

Experimental study on honeybee colonies: the test was conducted on a plantation far away from inhabited regions and sound, using ten contemporary Langstroth hives. Every nest had 6 boxes with waxy tops, 1000 grams of flowers and women (less than 1 year ancient), and nests were coated with a regular-sized web of black gauze (4 m wide, 6 m wide and 3 m tall). Within the optocoupler, five clusters of bees were positioned and put with an A symbol (Fig1) on the same panel. They were supplied with sugar solution (Pepsi) and pollen grains; but at the other hand, five colonies of bees were held on the same framework as an experimental group just outside of the seclusion under free flight circumstances and symbolized by letter (B), left to nourish instinctively in the farm land area, taking into account that the beekeeper leaves a volume of honey during the last crop before our tests started.

Procedure of nourishment with Pepsi: Two types of feeding were tested in this study, one in the natural ground, the other with Pepsi (soft drink) under lab conditions, 1.5 litres of Pepsi drink per day, poured over the feeding fluid in a plastic tub for 6 weeks with a piece of floating cork (figure 2) to allow the bees to start standing up during the stroking method. To perform the present survey, the adult phase of captive bee employees was selected.

Pepsi Component: Pepsi is produced of soda water, elevated corn syrups, and colour of caramel, sweetness, phosphoric acid, caffeine, citric acid and artificial flavours. A Pepsi can (12 fl ounces) contains 41 grams of carbohydrates (all sugar), 30 mg of fat, 0 grams of protein, 38 mg of caffeine, and 150 calories.^[61]

Honey harvesting: Honey was collected by the Parliament of Beekeepers in Saudi Arabia in compliance with the accepted standard enacted. Throughout collecting a beehive complete of fertilization, danger should be prevented so as not to cause them to harm. Selected images with six-pointed wax-sealed windows; because the sealing of bees for these beehives implies that the honey is fully focused, while collecting the uncovered wax, it will trigger rapid harm to the honey item, as it still includes elevated water levels.

Sample Collection and Preparation: Extracts of honey were collected using conventional techniques. All vials were freshly gathered in sanitized cans (labelled with numbers, location and collection date) and deposited at room temperature (regular 4 ° C fridge) until tested. Unwanted stuff such as wax pins, deceased bees and combs residues were separated by squeezing the components before analysing them through cling film.

Honey analysis: Two kinds of honey gathered from the present study have been sent to the beekeepers collaborative Honey Analysis Quality Laboratory (HAQL) in Al-Baha.

Biochemical Analysis: Using the AOAC techniques.^[14,15] approximate proportions of the honey extracts were calculated.

Procedures used in applied studies black animal experiment rats were chosen from the King Fahd Health research Centre at King Abdul-Aziz University in Jeddah. At such an era of 4-6 weeks the rats were chosen, and the weight varied from 70-86 g. At King Fahad Centre Labs in Jeddah, tests were performed in which they were put inside an excellently-ventilated space in personal laboratories. The rats were split into three communities, the comparison group was supplied to distortion with wet controlled bales comprising all the vital nutrients of experimental animals supplied by the Grain Silos and Flour Mills General Institution in Jeddah.

Team A was given 0.1 ml of Pepsi honey. The fifth band (B) received a quantity of 0.1 ml of artificial nectar honey. The injection was performed through the needle of experimental eating. In each band, thirty rats were used. Each cluster was split into six classifications, five replicates each composed of 5 rats. The mice were allowed to adjust for two days until the test started.

In the last week of the study, anesthetized anaesthesia was anesthetized to take blood samples and autopsy, with any alterations reported in the rats during the testing period. In the three teams, the rats were indeed measured.

All laboratory results have been registered; rat conduct modifications have been reported too instantly after therapy. The impacts of nutrition kinds were equated in

blood tests and pancreas, mean complete weight, and rat weight gain was collected in the three groups, mean total weight, and pancreatic weight gain was tracked too soon after the autopsy.^[50]

As explained in AOAC, in order to govern the contents of sucrose and reducing sugars, the Layne-Enyon technique was used.^[111] For this purpose, approximately 2.6 g of honey was weighed and poured into a 500 mL volumetric flask. Then in a 250 mL Erlenmeyer flask containing 7.0 mL of water and 15.0 mL of honey solution, 5 mL of standardized Fehling's solutions A and

B were transferred. After heating the Erlenmeyer flask, 1.0 mL of methylene blue (0.2%) was added to it. The titration process was conducted by adding the diluted honey solution until the indicator decolorized.

Inversion was used to decide the sucrose content by adding 10 mL of dilute HCl, 50 mL of diluted honey solution and water in a 100 mL volumetric flask. A water bath was used to heat the solution and then it was cooled and diluted to the mark. The Layne-Enyon method was then applied to acquire the sucrose content.



Fig 1: The beehives isolator



Fig. 2: a plastic container for 6 weeks with a piece of floating cork.

Statistical Analysis: Statistical studies were done by Statistical Package for Social Sciences (SPSS) version 22 (SPSS Inc., Chicago, IL, USA). The descriptive statistics had been applied to calculate means \pm standard error of mean (SE). Weight gain (%) was calculated as total body

weight at that week = initial body weight / initial body weight \times 100. Pancreas index was calculated as pancreas weight / final body weight \times 100. One Way ANOVA test followed by least significant difference (LSD) test was performed to find out the significant difference

between groups. Unpaired student “t” test was used to compare content of honey A and B. P values of ≤ 0.05 were considered significant.

Results: Sucrose and moisture content were significantly higher in Honey B compared to Honey A (P =0.001 for both). While, glucose + fructose, glucose, fructose, hydroxyl ethyl flurfural and diastase were lower in Honey B compared to Honey A (P =0.001 for all) (Table 1 and Figure 3).

The initial total body weight was significantly higher in Honey outside B1 group compared to Honey control group (P =0.025). At the 3rd week, the total body weight in Honey inside A1 group was significantly higher than Honey control group (P =0.040). At the 4th week, the total body weight in Honey outside B1 group was significantly lower than Honey control group (P =0.013) (Table 2 and Figure 4).

The weight gain in Honey outside B1 group was significantly lower than Honey control and Honey inside

A1 groups at 1st week (P =0.001, P =0.029), 3rd week (P =0.047, P =0.019) and 4th week (P =0.001, P =0.022). At the 2nd week, the weight gain in Honey outside B1 group was significantly lower than Honey inside A1 groups (P =0.049) (Table 3 and Figure 5).

The pancreas weight was significantly higher in Honey inside A1 group compared to Honey control and Honey outside B1 groups (P =0.018, P =0.020). Meanwhile, there were no significant changes between pancreas index in different studied groups. (Table 4 and Figures 6& 7).

The serum insulin level was significantly lower in Honey inside A1 group compared to Honey control and Honey outside B1 groups (P =0.001, P =0.001). Meanwhile, the serum glucose level was significantly higher in Honey inside A1 group compared to Honey control and Honey outside B1 groups (P =0.001, P =0.001) (Table 5 and Figures 8 & 9).

Table (1): Comparison of components of honey in different types.

Parameters \ Type	Honey A	Honey B	Significance
Sucrose	0.89±0.01	2.60±0.15	¹ P= 0.001***
Sum(glucose+fructose)	72.98±0.05	61.36±0.94	¹ P= 0.001***
Glucose	34.28±0.10	28.64±0.54	¹ P= 0.001***
Fructose	39.00±0.44	31.60±0.35	¹ P= 0.001***
Hydroxy ethyl Flurfural (HMF)	58.06±0.70	49.38±0.59	¹ P= 0.001***
Diastase	24.14±0.46	20.18±0.48	¹ P= 0.001***
Moisture content	13.70±0.23	16.59±0.29	¹ P= 0.001***

Data expressed as mean +/- standard error. ¹P: significance versus honey A. Significance was made using unpaired student “t” test.

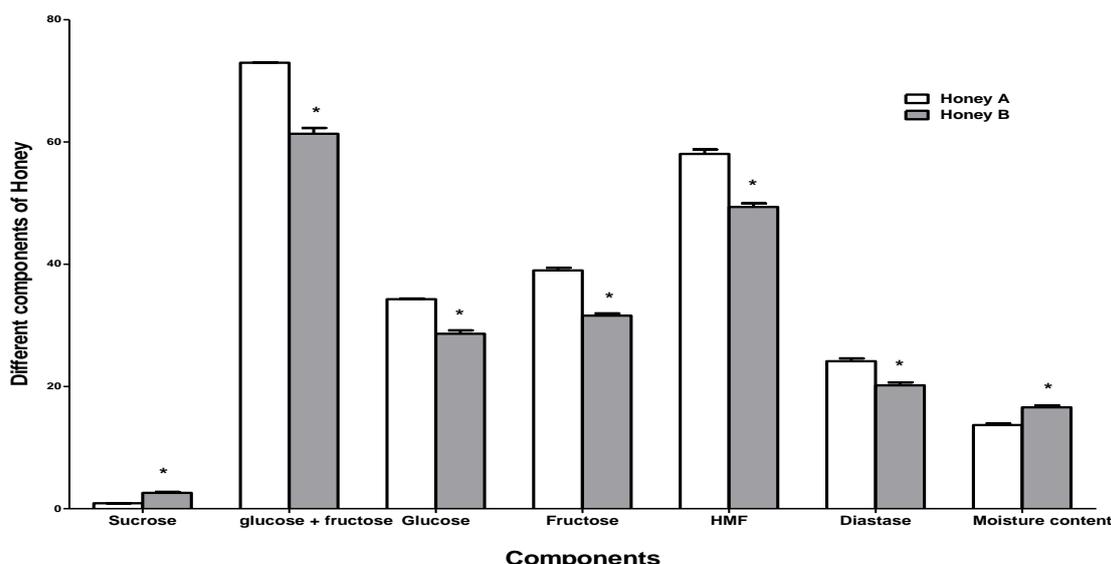


Figure (3): Comparison of components of honey A and honey B. Data expressed as mean +/- standard error. * : significance versus honey A. Significance was made using unpaired student “t” test.

Table (2): Comparison of total body weights (grams) at different weeks in different studied groups.

Groups	Honey control (n=10)	Honey inside A1 (n=10)	Honey outside B1 (n=10)
Body weight			
Initial (grams)	71.20±5.18	77.30±4.52	86.20±3.57
Significance		¹ P= 0.343	¹ P= 0.025* , ² P= 0.171
At 1st week (grams)	103.00±5.44	114.13±8.16	112.89±5.74
Significance		¹ P= 0.232	¹ P= 0.271, ² P= 0.895
At 2nd week (grams)	118.40±5.23	141.38±10.28	137.33±8.97
Significance		¹ P= 0.058	¹ P= 0.103, ² P= 0.735
At 3rd week (grams)	140.60±5.56	172.63±12.37	156.56±12.89
Significance		¹ P= 0.040*	¹ P= 0.276, ² P= 0.299
At 4th week (grams)	211.60±5.64	200.88±13.38	169.67±14.26
Significance		¹ P= 0.511	¹ P= 0.013* , ² P= 0.070

Data expressed as mean +/- standard error. ¹P: significance versus honey control; ²P: significance versus

honey outside A1. Significance was made using one way ANOVA test.

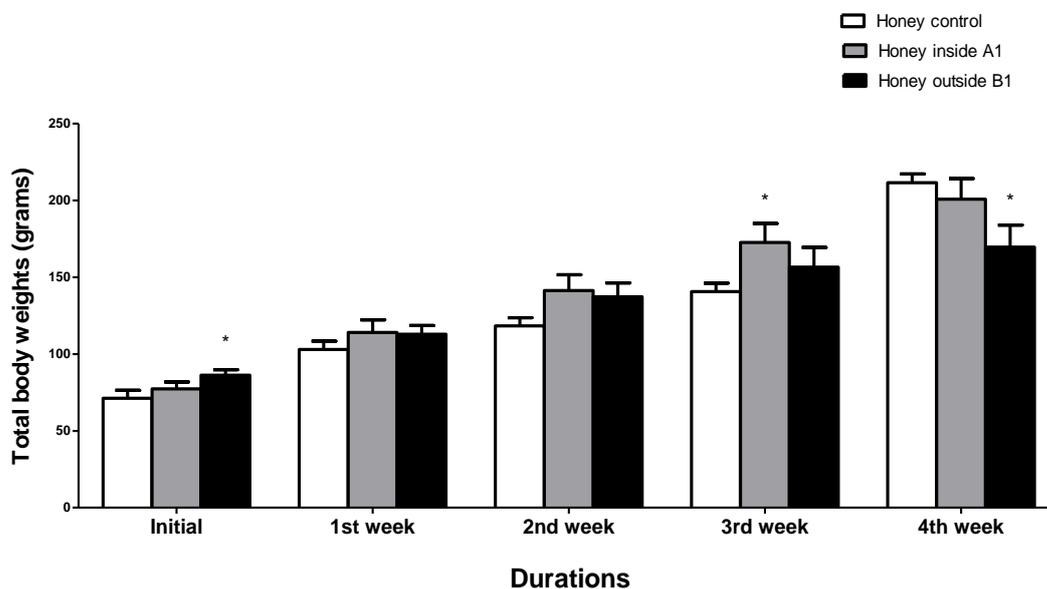


Figure (4): Comparison of total body weights (grams) at different weeks in different studied groups. Data expressed as mean +/- standard error. *: significance versus honey control. Significance was made using one-way ANOVA test.

Table (3): Comparison of weight gain (%) at different weeks in different studied groups.

Groups	Honey control (n=10)	Honey inside A1 (n=10)	Honey outside B1 (n=10)
Weight gain			
At 1st week (%)	46.32±2.62	38.51±3.64	26.66±4.21
Significance		¹ P= 0.129	¹ P= 0.001*** , ² P= 0.029*
At 2nd week (%)	69.09±4.53	71.81±6.42	53.65±7.10
Significance		¹ P= 0.753	¹ P= 0.074, ² P= 0.049*
At 3rd week (%)	102.27±9.38	109.83±7.18	74.74±11.12
Significance		¹ P= 0.583	¹ P= 0.047* , ² P= 0.019*
At 4th week (%)	209.09±20.23	144.86±9.22	89.08±12.34
Significance		¹ P= 0.008***	¹ P= 0.001*** , ² P= 0.022*

Data expressed as mean +/- standard error. Weight gain calculated as body weight at that week – initial body weight / initial body weight X 100. ¹P: significance versus honey control; ²P: significance versus honey outside A1. Significance was made using one way ANOVA test.

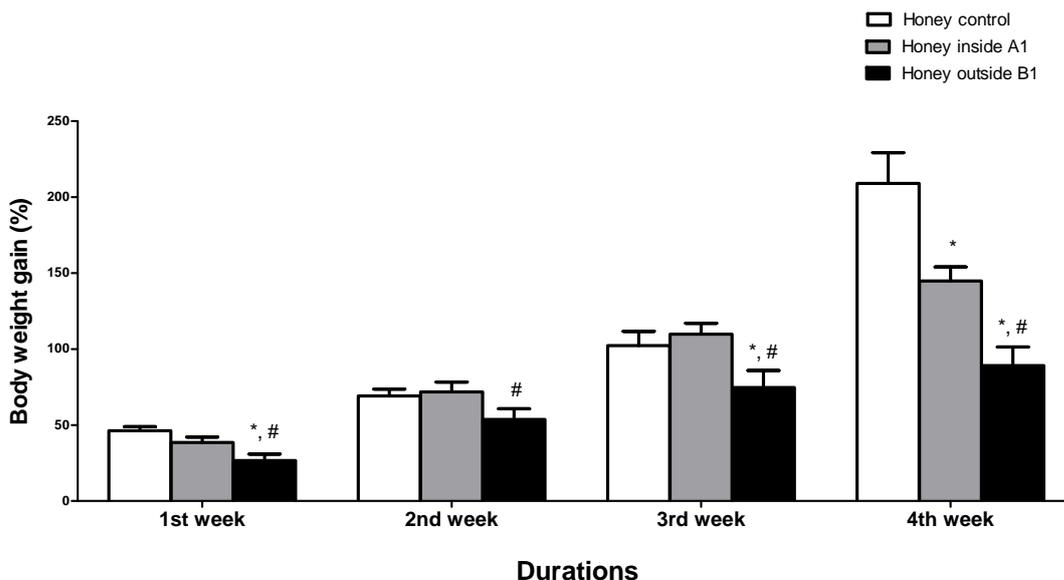


Figure (5): Comparison of weight gain (%) at different weeks in different studied groups. Data expressed as mean +/- standard error. *: significance versus honey control; #: significance versus honey outside A. Significance was made using one-way ANOVA test.

Table (4): Comparison of pancreas weight (grams) in different studied groups.

Variable \ Groups	Honey control (n=10)	Honey inside A1 (n=10)	Honey outside B1 (n=10)
Pancreas weight (grams)	0.80±0.06	1.39±0.27	0.82±0.06
Significance		¹ P= 0.018*	¹ P= 0.948, ² P= 0.020*
Pancreas index (%)	0.38±0.03	0.61±0.17	0.72±0.02
Significance		¹ P= 0.089	¹ P= 0.469, ² P= 0.310

Data expressed as mean +/- standard error. Pancreas index calculated as pancreas weight / final body weight X 100. ¹P: significance versus honey control; ²P:

significance versus honey outside A1. Significance was made using one-way ANOVA test.

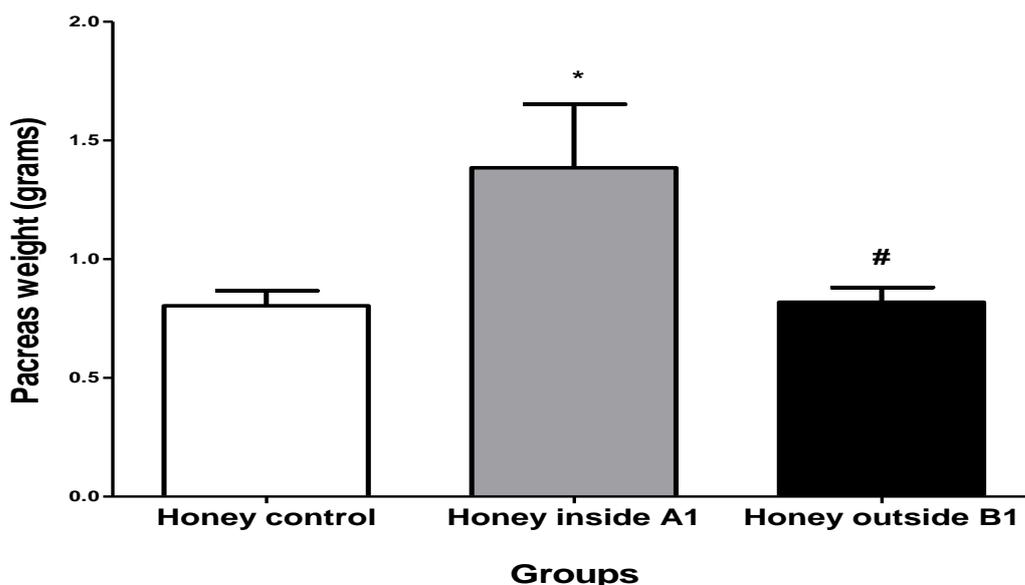


Figure (6): Comparison of pancreas weight (grams) in different studied groups. Data expressed as mean +/- standard error. *: significance versus honey control; #: significance versus honey outside A1. Significance was made using one way ANOVA test.

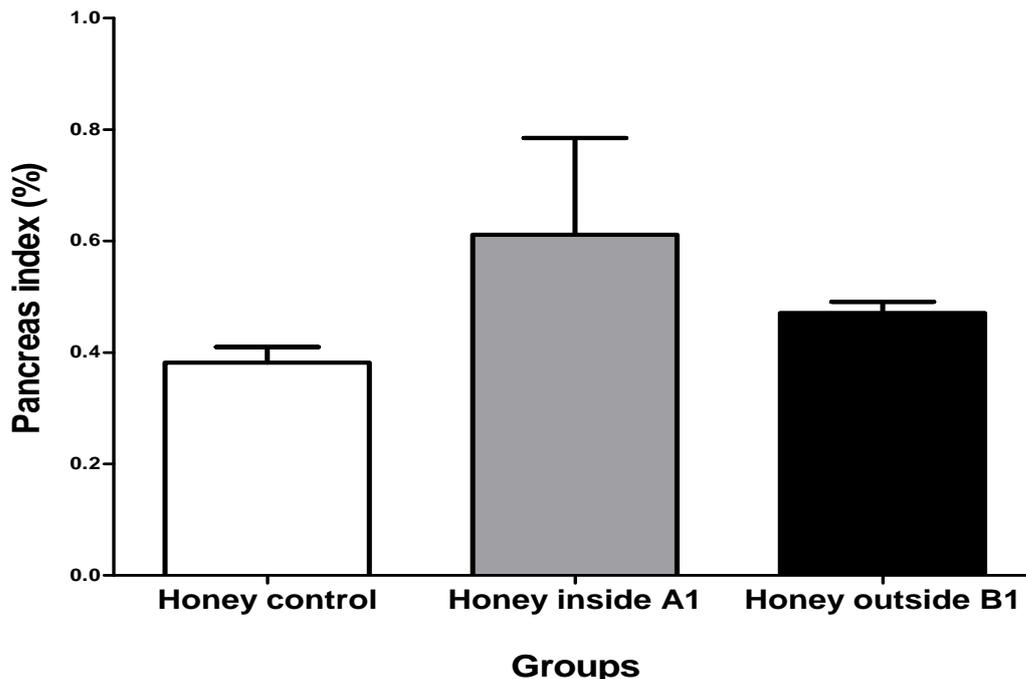


Figure (7): Comparison of pancreas index (%) in different studied groups. Data expressed as mean +/- standard error.

Table (5): Comparison of serum levels of insulin (U/L) and glucose (mg/dl) in different studied groups.

Groups	Honey control (n=10)	Honey inside A1 (n=10)	Honey outside B1 (n=10)
Insulin (U/L)	15.10±1.20	1.91±0.18	16.67±1.39
Significance		¹ P= 0.001***	¹ P= 0.308, ² P= 0.001***
Glucose (mg/dl)	88.00±2.21	211.80±8.57	86.40±2.69
Significance		¹ P= 0.001***	¹ P= 0.834, ² P= 0.001***

Data expressed as mean +/- standard error. ¹P: significance versus honey control; ²P: significance versus honey outside A1. Significance was made using one way ANOVA test.

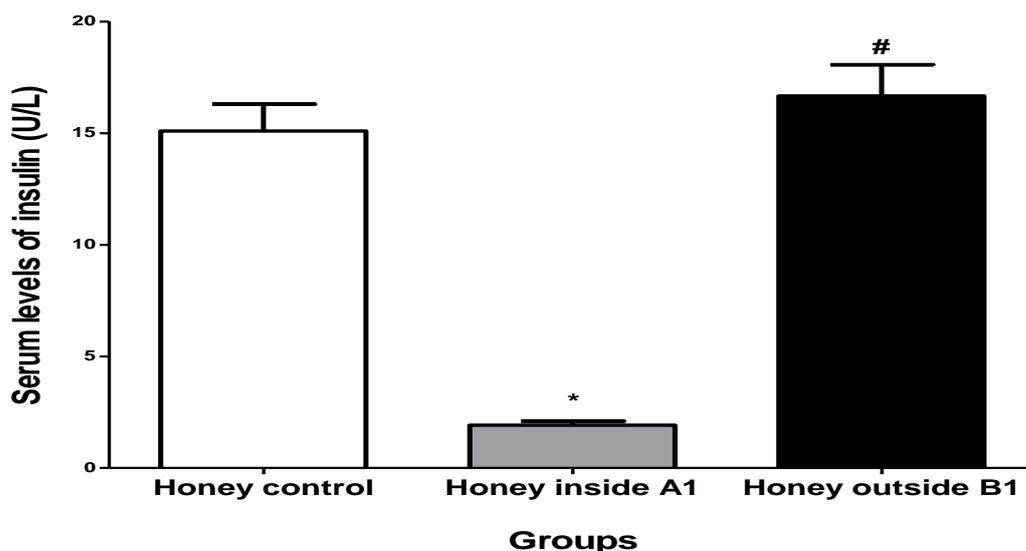


Figure (8): Comparison of serum levels of insulin (Uiu/l) in different studied groups. Data expressed as mean +/- standard error. *: significance versus honey control; #: significance versus honey outside A1. Significance was made using one way ANOVA test.

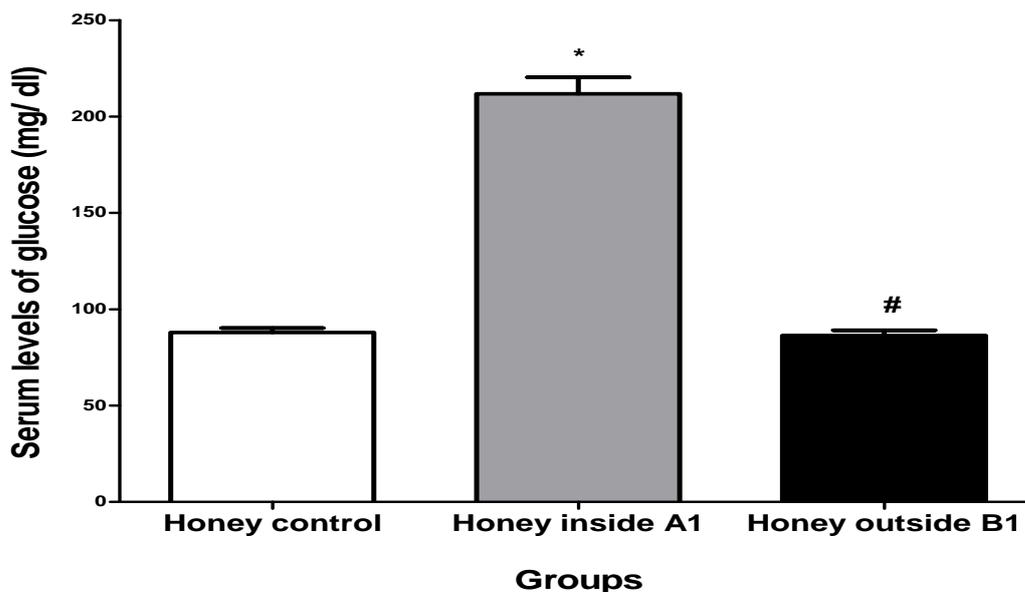


Figure (9): Comparison of serum levels of glucose (mg/ dl) in different studied groups. Data expressed as mean +/- standard error. *: significance versus honey control; #: significance versus honey outside A1. Significance was made using one way ANOVA test.

DISCUSSION

Honeybees get their name from their ability to produce a natural sweet substance known as honey, which is extracted by them from the plant flower nectars and honey dew. A reputable global expert is responsible for determining the significance of Honey analysis, as he is equipped with the ability to recognise the requirements of honey and bee products. A genuinely authentic analysis procedure is required for the royal jelly, bee pollen, propolis and beeswax.

The artificial sweeteners such as high fructose corn syrup (HFCS) that are commonly used in soda and soft drinks tend to sweeten more than ordinary sugar. Another reason for excessive usage of HFCS is that it's cheaper. HFCS are six times sweeter than ordinary sugar and very cost effective as well, which makes it the most commonly used sweetening agent in soft drinks.

As stated by Codex Alimentarius Commission (CAC), Honey tends to have average moisture of not more than 20.0 g/100 g.^[28] There were low moisture contents observed in samples of group A and B contents (13.70±0.23& 16.59±0.29) respectively, which were all in limit of 20.0 g/100 g in accordance to global standards. As declared by White and Doner, the values lie between the ranges of moisture contents.^[137] Other researchers have also produced similar results to these.^[136,100,12,93] In order to determine the honey shelf-life, it is significantly important to consider moisture content for the quality measurement.^[27,28] In the global trading of honey, this is the only vital composition criterion that is used for honey standard.^[26] The moisture in honey hold key importance because of the link between honey water content and yeast count; at 17.0

g/100 g moisture (humidity) and there is extremely low fermentation danger due to reduced yeast count.^[27] Hence, high water content in honey tends to ferment it.^[26] According to the Codex Alimentarius Commission.^[44] and EU Commission.^[60] a maximum value of 20.0 g/100 g was declared as international standard for honey moisture contents.

This study analysed the fructose contents in honey samples A and B to be within an average (39.00±0.44&31.60±0.35) respectively. However, various samples showed different average fructose contents from each other, (group B) is found to be within the range of values declared by other scientists, while group(A) surpassed the standard limit adopted.^[137,12,93,142]

Similarly, there was a clear difference between the glucose contents of honey samples taken from the tested samples. A and B samples had glucose content (34.28±0.10, 28.64±0.54), which was (P<0.0001) notably lower than the fructose contents. This test result affirms fructose being the key sugar in all analysed samples and also corresponds with the previous observation of White and Doner.^[137] The main two types of sugars in honey are fructose and glucose, and though they have no particular limits to their individual values; however their sum (Fructose+glucose) has been fixed at an international standard value of ≥ 60 g/100 g for honey by the Codex Alimentarius Commission. This study affirms that the sum of fructose and glucose for the honey samples have their values conforming to the limit set up by the international standards; i.e., 60g/100 g and above. They study of White and Doner.^[137] shows that what makes honey different from the commercial invert sugar is its fructose dominance over glucose. The scale of sugar in honey mainly depends on sugars found in

nectar and enzymes found in the bee and nectar.^[137,28,142] The primary sugars found in all honey samples are fructose and glucose, and sign of a high quality of honey is that it has higher fructose content than glucose.^[49] The ratio of fructose and glucose determines the honey's ability to crystallize. According to White and Doner,^[137] despite the fact that glucose content is less in honey, it is due to glucose that honey crystallizes and forms granulates as it is less soluble in water than fructose. High ratio of fructose/glucose keeps the honey in liquid form. Honey crystallization becomes slower when the fructose/glucose ratio is more than 1.3 and is faster with ratio below than 1.0.^[12] In order to correctly predict the honey crystallisation, the glucose/water ratio is more appropriate as compared to fructose/ glucose because honey contains others sugars (sucrose, maltose, furanose, etc.) and insoluble substances (like dextrin, colloids, etc.) as well, which can affect the crystallization process. When the glucose/water ratio is lower than 1.3 then honey crystallization is either extremely slow or zero, and the crystallization process is instant with ratio higher than 2.0.^[12] Glucose being the key ingredient in honey instantly crystallizes from honey solutions as a monohydrate.^[137] This is usually observed when honey's moisture level drop below a specific level, like when the moisture content is extremely low. As previously stated, the honey sample with (G/W) ratio less than 1.7 is non-granulating as compared to samples with ratios ≥ 2.1 that quickly granulates. As stated by Manikis and Trasivoulou,^[94] honey granulation can be effectively determined by glucose level and the G/W ratio is regarded as one of the best indicators prediction of granulation tendencies in honey samples. Hence, the granulation tendencies in honeys can both be predicted and controlled by G/W ratio.

According to the Codex Alimentarius Commission, an international standard for a good quality honey is having no more than 5 g/100 g of sucrose content. In Buba *et al.*,^[36] study showed that honey samples under study had sucrose contents within the range of 0.53 to 3.29 and an average of 1.84 ± 0.79 g/100 g. the study affirmed that sucrose values obtained from honey samples were in accordance to the international standards (table1, Fig3). Moreover, White and Doner,^[137] stated that despite sucrose being an active splitting enzyme (sucrase, glucosidase) in honey, the sucrose level never goes down to zero. The examination of sucrose contents in this study are within the range of values reported for Argentine and Turkish,^[42,131] Venezuelan,^[36] American,^[137] Algerian,^[93] Pakistani,^[142] and Spanish.^[38]

As far as tolerance to low temperature is concerned, the worker bees' ability to tolerate low air temperatures is influenced by the feeding choices. In order to survive cold temperatures, the honeybees need a source of energy such as, honey or other alternative to generate energy. The results of current study show that natural nectar is better than soft drink (Pepsi). The natural nectar feeding choice appears to be a more energy source for

the bee workers. Despite the fact that there is more sugar or sucrose in Pepsi, it still portrayed lower ability to increase the bees' tolerance towards low temperature over time. Another proof of feeding effect is the natural nectar as an energy source even in severe weather. The bees that fed on Pepsi were unable to take up required nutrients as energy source as they were not included. These bees also had lowest rate of survival. Moreover, their energy was used up rapidly and they became motionless within short time. As compared to bees fed on Pepsi, the bees on nutrition natural nectar choices recovered quickly. The Pepsi feeding bees had slower recovery rate and only few of them recovered. One reason could be the lack of essential dietary supplements in Pepsi, whereas the nectar feeding bees could produce enough amount of energy to recover. The natural feeding bees were also found to have higher survival abilities in the cold conditions, whereas the Pepsi feeding bees had lower chances of survival. Apart from consumption of food, the ability of bees to use that food as energy source also effects their tolerance towards low temperature. The study results show that survival periods of honeybees also depended on their feeding choices. It is important to further study the better feeding choices of bees through experimentation and statistically so that the key variations between the two feeding types could be determined. The honey and sugar (sucrose) combination was found to be more effective, which was followed by only honey or only soft drinks in liquid or natural nectar form.^[36]

On the contrary, Barker & Lehner,^[20] who found that caged bees were able to survive longer when they fed on sucrose syrup than honey, which conflicted with the observations of the present study, workers fed on natural nectar fields survived longer than those fed on Pepsi syrup were recorded. This should be explained through accurate statistical studies under experimental condition in another studies. Basically, honey from natural sources was able to provide worker bees with adequate energy for a longer period than other offered feeding on Pepsi. This could be explained by the presence of natural nectar rich with sources of sucrose as an energy source. Also, in natural nectar may increase the ability of worker bees to absorb nutrients from it over Pepsi drink.

HMF can be found in low amounts in honey, fruit-juices and UHT-milk. Here, as well as in vinegars, jams, alcoholic products or biscuits HMF can be used as an indicator for excess heat-treatment. For instance, fresh honey contains less than 15 mg/kg—depending on pH-value and temperature and age (Codex1998),^[45] and the codex Alimentarius standard requires that honey have less than 40 mg/kg HMF to guarantee that the honey has not undergone heating during processing, except for tropical honeys which must be below 80 mg/kg.

It can be found in glucose syrup HMF can form in high-fructose corn syrup (HFCS), levels around 20 mg/kg HMF were found, increasing during storage or

heating(Codex1998).^[45] This is a problem for American beekeepers because they use HFCS as a source of sugar when there are not enough nectar sources to feed honeybees, and HMF is toxic to them. Adding bases such as soda ash or potash to neutralize the HFCS slows the formation of HMF (Codex1998).^[45] This major honey quality factor is an indicator of honey freshness and overheating. In fresh honeys there is practically no hydroxymethylfurfural (HMF), but it increases upon storage, depending on the pH of the honey and on the storage temperature. Some European bee federations (Germany, Belgium, Italy, Austria, Spain) market a part of their honey as 'quality honey', having a maximum of 15 mg/kg. In international trade, a maximum value of 40 mg/kg has proven satisfactory. In long-term routine honey control at the IHA during the last 10 years, more than 90% of the raw honey samples (30 000) and more than 85% of the retail honey samples (2000) had less than 30 mg HMF/kg. The Codex proposal gives a maximum of 60 mg/kg. The proposal for a higher maximum value is based on the experience that HMF increases on honey storage in warm climate countries.^[94] The latest EU standard proposal demands a maximum of 40 mg/kg, as under European conditions this standard has proven to be valid. Here too, as in the case with diastase, there is an important difference between the two norms: while the Codex norm refers to honey after processing and blending, the EU norm is valid for the whole life of the retail honey. This means that the EU norm is much more severe than the Codex norm, as HMF is expected to increase upon storage.

Hydroxymethylfurfural (HMF), formed from the acid-catalyzed dehydration of Hexose sugars, especially fructose, and formed in honey as a result of heat treatment or storage. High HMF levels must also be considered as a risk in the feeding of invert sugars or HFCS. A good estimate of the toxicity of HMF was published by Jachimowicz and El Sherbiny,^[78] Sugar solutions containing 150 ppm HMF result in 58.7% mortality within 20 days 2.2, the high mortality of bee in group B maybe reversed to I, May be supported by another study,^[36] whereas solutions containing 30 ppm cause a mortality of only 15.0%, which was not significantly different from the controls (12.5%). HMF levels of 30 ppm can therefore be regarded as safe for bees. Recently, the HMF content of commercially available HFCS was determined to be between 3.1 and 28.7 ppm by LeBlanc et al.^[90] They also demonstrated that HFCS stored at 40 °C for 69 days may reach 250 ppm, a concentration that significantly reduced longevity compared to HFCS with lower concentrations of HMF (57–200 ppm). The authors rightly opposed a comparison of bee mortality between HFCS and sucrose, but bees in their experiment survived better on sucrose.^[31] The current study showed (58.06±0.70& 49.38±0.59) in group (A) followed with (B) respectively, with consideration the storage period did not exceed four days at 4°C, the high level of HMF, despite appropriate

storage conditions, may be due to the components of feeding substances in group A.

It is not expected that HMF levels were increased substantially to cause adverse impacts on worker bees tested. Providing bee colonies during cold air temperature with natural nectar showed the ability to enhance worker tolerance to cold temperatures conditions is worthy of being mentioned that the nutritional status of the colonies can not only impact the activity of bees but as well as their long-term behavioral development.^[121] The form of the feeding type (natural nectar or soft drink) impacted the consumed amounts. It was clear the bees were able to consume. Lower activity and high mortality, in group (B) in this study were recorded.

Honey can contain a few different enzymes. Some of these are introduced by bees, and some are found in the nectar. As with most aspects of honey, different nectar/honey sources have widely varying enzyme activity. Typically, enzymes are proteins of complex structure that catalyze a specific chemical reaction. They are sensitive to heat, visible and UV light and other forms of energy such as microwaves.^[14]

In simplistic terms, this enzyme is responsible for converting starch to dextrins and sugars and is introduced into honey by the bees. Its main point of interest is as an indicator of heating - much like HMF and is usually used in conjunction with HMF. It is measured with an empirical scale - the Gothe scale. Some honeys are naturally very low in Diastase. The Codex standard has a minimum of 8 on the Gothe scale for Diastase and a special category for honeys low in Diastase of 3. However, in the case of honeys low in Diastase, the HMF must not be more than 10 mg/kg (compared with the more normal 80 mg/kg).

Of all the enzymes in honey, Diastase and Invertase have received the most attention. They are introduced to honey by bees but their presence [in fresh honey] is variable. Factors that affect their presence are thought to be nectar composition and concentration, the age of the bees, and the intensity of the nectar flow. e.g. an intense flow of nectar with a high concentration usually yields low values for diastase and Invertase activity.

Diastase: Honey samples of both group A &B showed (24.14±0.46) (20.18±0.48) respectively. All tested samples were within regulatory requirements because the DN is greater than 8. The highest value activity of α -amylase has a non-commercial fewer honey derived directly from the beekeeper and stored at 4 °C. Honey samples in different study were stored at room temperature, are characterized by slightly lower DN (17.6). This difference may indicate little effect of storage conditions on the value of DN in fresh hone. According to,^[36] the diastase activity of honey samples from Perari Forest was decreased 81.55% during the

initial eight months and 78.43% by the end of sixteen months. The same behavior of loss of diastase activity was also noticed by White and Subers.^[105,106,107] Sancho et al.^[117] studied the effect of storage for two years on diastase activity by keeping the honey samples at 20°C. Cervantes et al.^[39] first heated the honey at 55°C and then stored for three and half months at 26°C. Both scientists observed depletion in diastase activity of honey under different storage conditions.

When interpreting diastase results, one should take into consideration that certain unifloral honeys have a naturally low diastase activity. Although the minimal requirements for diastase activity in the Codex and the EU drafts are the same, in practice there is an important difference between the two: while the Codex norm refers to honey after processing and blending, the EU norm is applied to the whole life of the retail honey. This means that the EU norm is more severe than the Codex norm, as diastase activity is expected to diminish upon storage. Based on the above, consideration should be given to storing honey in Saudi Arabia, which is characterized by a summer of up to 55°C in some areas.

Applied study of feeding rats on honey: Honey is a natural product produced by honeybees. Honey contain unique and distinct types of phenolic and flavonoid compounds of variable biological and clinical importance. Honey is one of the most effective natural products used for wound healing. In this review, the traditional uses and clinical applications of honeybee and such as antimicrobial, antioxidant, antiinflammatory, anticancer, antihyperlipidemic, and cardioprotective properties; the treatment of eye disorders, gastrointestinal tract diseases, neurological disorders, and fertility disorders and wound healing activity are described.^[84,96,62,116,67] Honey has held a place of importance in traditional medicine,^[3] for ages,^[81,103] For many years, honey has been a pivotal player as an antioxidant, and it has been reported that honey can be used as a hepatoprotective and cardioprotective agent.^[55,54,57] In addition, honey has protective effects against gastrointestinal ailments,^[53] Furthermore, honey is traditionally used as an anti-diabetic,^[58,65] and hypolipidemic agent (Adnan et al., 2011) and to ameliorate thyroid disturbances.^[3]

Beekeepers usually supply their colonies with alternatives to nectar (i.e. sugar feeding, and soft drink) during dearth periods of the year, especially cold times of winter. Its already been proved that **Soda/Soft Drinks** can cause serious health problems. It was found that they contain artificial sweeteners like high fructose corn syrup (HFCS) that sweetens more as compared to ordinary sugar. HFCS were already under the radar due to their direct effects on liver disease, obesity and diabetes.^[36,97,91]

Health problems caused by HFCS, obesity, fatty Liver disease, diabetes.^[22] Though it is harmful in every aspect

for human body, its being used in vast amount by the drink companies and they are saving much money this way. Despite of such evidences, media coverage and laboratory reports, HFCS are still being used on a large scale.

Obesity in group (A) may be attributed to the adoption of bees in the manufacture of food on the components of Pepsi and supported by many research that the drinking permanently leads to increased obesity leading to diabetes, some research said that obesity inhibits insulin performance of work according to Mentioned in.^[130,79,83] A decrease in the weight of rats fed on natural nectar honey was observed with the level of insulin and blood sugar closed to the levels of the control samples indicating that natural honey may be considered a healthy diet that helps to lose weight safely enhances the health of the body. The increase in the weight of the control samples is attributed to progress in age associated with a normal increase in weight and volume. The increase in the weight of the pancreas in rats fed on honey's Pepsi is attributed to pancreatitis due to the incidence of rat's diabetes and the inability of cells to produce insulin.^[130]

One of the most noticeable applications of honey is in reducing cholesterol levels in hyperlipidemic patients. For example, the continuous administration of 75 g of honey dissolved in 250ml water for 15 days significantly reduced lipid levels.^[10] Another study by Yaghoobi et al.,^[140] reported the effects of honey on fasting blood glucose (FBG), body weights, which support the results of this study, agreed with the results has been recorded in the current study. In addition to suggesting that regular honey consumption has the health benefits of glycemic control and an improvement in the lipid profile, which directly or indirectly leads to a reduction in the occurrence of diabetes disease.^[41] Many studies have examined the wound healing effects of honey.^[75] The wound healing activity of honey on experimental mice, which received topical application of honey, has been positive. Histopathology findings showed significant improvement in granulation tissue thickness and open wound size, the study also suggested that the topical application of honey to wounds may exhibit a wound healing capacity, in a human study of 59 patients, honey was found to improve wound healing.^[23] The results of this study show the importance of the source of honey chosen for treatment especially for diabetic people, because of its consequences of complications such as non-fusion wounds that often lead to gangrene and amputation of organs. Wherefore, care should be taken when choosing the honey, according to the observation of our study, honey from soft drinks sources should be avoided.

The antihyperglycemic effects of honey in rabbits with chemically induced diabetes have been confirmed. One study found that different doses of honey (as low as 5ml/kg) produced a significant reduction in blood

glucose levels and other related parameters, which agreed with this study. The study indicated that even at low doses (5ml/kg), honey may be a good alternative to sucrose as a natural sweetener for diabetic patients.^[5] Honey and its components were found to have several health benefits with long-term usage. Honey showed beneficial effects in one report, including weight improvement and reduction in blood glucose levels.^[109] Honey contains a high concentration of fructose, a monosaccharide capable of elevating blood glucose levels through oral absorption. It is therefore a paradox that researchers and nutritionists have encouraged the use of honey as a nutrition supplement in diabetic individuals.^[2,88]

All these studies documented the therapeutic benefit of honey in various diseases, including obesity and diabetes. Therefore, it is essential that honey should match international quality standard. In the current study Statistical analysis of the rates Weight showed increased in body weight, and weight gain in group A followed by group B, finally the control group respectively (table 2,3, Fig 3,4). Indicating that the daily consumption of (honey's Pepsi) could cause obesity leading to diabetes, The results of this study support hypothetical relationship between feeding on honey's Pepsi and diabetes, according to the results of the blood glucose and insulin of rats in three different groups (Pepsi, which showed an increase in the level of blood sugar associated with decrease in the level of insulin secreted, compared to the control group, while no disorder were recorded in the measurements of glucose and insulin in the group feeding on the natural nectar's honey were recorded (table 5, fig 7) which supports the assumption that it is safe to be consumed for the purpose of nutrition and treatment.

Simple sugar (glucose) is the primary and safe source of physiological processes in the brain, many studies showed that it **effects on brain function** as well. **High amounts of HFCS disturbs the insulin activity in brain which maintain the sugar for energy process.** Brain cells were not able to properly signal each other in order to rectify thoughts. Therefore, rats who were given HFCS got troubled in finding their way to exit. It is being deduced that; similar memory problems can occur in humans like memory loss. But when HFCS were thoroughly tested by the scientists of University of California, they found its direct impact on Brain functioning as well.^[122] The experiment was performed on rats due to their brain chemistry much similar to humans. Two categories of rats were examined, those with HFCS given and those not given and were tested in a maze to observe their thinking capability. It was found that rats that had not been given HFCS were much better in finding their way than the rat given HFCS. The scientists then conclude that, A recent study on Tualang honey from Malaysia in the context of neurodegenerative disorders reported that honey may have significant activity against chronic cerebral hypoperfusion, which is

one of several factors contributing to Alzheimer's disease.^[122] Several studies have confirmed the beneficial effects of honey on fertility as well as in ameliorating the hormones related to fertility.^[108]

All these previous dangerous results, which have recorded the possibility of fructose sugar in causing of Alzheimer, the global disease that permeate in our country with its healthy (physically, psychologically) and economic problems on the patient, families and countries supporting health and treatment programs. Supports the keenness of developed countries producing honey to establish standards to ensure quality, by preventing the use of soft drinks and processed foods rich in fructose in the feeding of bees in the beekeepers to reduce all kind of health damage expected.

Many life processes of vertebrates can also be found in insects. Insects are the more ancient group it is probably more correct to refer to the insect-type hormones that are present in vertebrates. The operation of such essential systems as protein synthesis, muscle contraction, and cell metabolism do not differ significantly between us and insects. An indication of the unity that exists between insects and vertebrates is best described by the example of the female rabbit flea that depends on the hormones circulating in the blood of its pregnant female vertebrate host in order to reproduce. It should therefore not come as a surprise that many hormones previously isolated from vertebrates are also present in insects, although their parallel functions have yet to be fully determined, and important differences in amino acid structure question their true homologies with vertebrate peptides. Insulin controls the rate of glucose transport across cell membranes and relays nutritional information that organisms use to regulate their growth. Its functions involving the regulation of growth have been largely conserved in insects. Bombyxin, the small PTH, shares a significant sequence homology with the A chain of vertebrate insulin. In *Bombyx mori*, the melanization-reddish coloration hormone shares some sequence homologies with insulin-like growth factor II. Gastrin and cholecystokinin (CCK) are related peptides that respectively mediate an increase in the secretion of acid in the vertebrate stomach and cause the gallbladder to contract. In insects, the sulfakinins show structural and functional similarities to gastrin and CCK. Somatostatin is a master hormone in vertebrates that controls the release of many other hormones. Somatostatin-like peptides have been found in such diverse insects as crickets, hoverflies, and locusts. Adipokinetic hormone regulates lipid mobilization from the fat body in insects and has homologies with vertebrate glucagon. Insect tachykinins stimulate visceral muscles and are structurally homologous with vertebrate tachykinins that are involved in processes as diverse as salt balance, sensory processing, and gut motility. FMRFamide-related peptides are found in many vertebrates and invertebrates. They have been isolated from insects where they stimulate muscle contraction and frequency

of heartbeat, and they regulate behavior in female mosquitoes. Melatonin is produced by the vertebrate pineal gland during the scotophase and causes drowsiness in humans. Its occurrence in the compound eyes of locusts and in a variety of other insects suggests it may also be involved in photoperiodism in invertebrates. Melatonin levels in several insects show a circadian rhythmicity, and the hormone has been implicated in the circadian release of PTH in cockroaches. Octopamine and tyramine are the invertebrate counterparts to the adrenaline and noradrenaline of vertebrates. They are classified as biogenic amines and are the only nonpeptide hormones found exclusively in invertebrates. Functioning variously as neurohormones, neurotransmitters, and neuromodulators, they regulate an abundance of physiological processes in insects, including the behaviors involved in the flight-or fight response, energy metabolism, learning and memory in bees, muscle contraction, and the sensitivity of sensory neurons.^[87]

Some research has supported the effect of nutrition type on bee weight and the possibility of causing obesity such as in vertebrates and mammals. The quantity and quality of both natural energy (manna, nectar, honey) and protein (pollen, pasture) are directly influenced by the climatic conditions of the active bee season.^[59] In addition to the characterization of food ingredients, bee nutrition also analyzes how nutrients and energy are used in the body, as well as their efficiency in different processes and production of bee colonies.^[59] In times when bees lack these natural food sources or if they are insufficient, beekeepers administer different food recipes to colonies just to provide them with the energy and nutrients needed to develop them. Thus, energy syrups have the role of substituting nectar, while pollen can be replaced with beer yeast, soy flour, powdered food consumption.^[33] The method of isolating bees in cages and administering different food recipes may be aimed at elucidating some problems regarding bee psychology, toxicology or parasitology,^[68] as well as determining certain productive parameters of bee colonies.^[136] This method of feeding isolated bees from colony is advantageous because it reduces the working time and this is a determining factor in the study of effects on bees of chemicals used in agriculture such as pesticides, neonicotinoids,^[64] Regarding the evolution of some parameters of their quality assessment, individuals within a bee family react differently to some additional food sources than isolated bees in cages,^[9] the presence of pollen in nature increases the lifetime of bees in the field, while its use in feeding bees in cages causes negative effects on this indicator,^[104] These different results of the pointers obtained in the field and those obtained in the case of isolation of the bees are also found in the case of the administration of the energy recipes. For example, in some experiments, honey, although it is natural energy feed, determined higher bee field lifetime values than those obtained from sugar syrup administration, while

administration on caged honeybees determined higher values,^[1]

Eşanu et al,^[59] obtained from their study that caged honeybees react differently to bees kept in the field (normal apiculture) in terms of body weight and food intake. They noticed that lots fed only with energy syrups recorded higher values of the bee's average weight at the end of the control period than those to which we added protein to food. These results are contrary to those obtained in the normal beekeeping when it is recommended to administer proteins during the autumn period for the deposition of adipose tissue necessary for crossing the cold season.

After analyzing the results of the energy-fed batches, we noticed that honey (the natural energy food source for bee) determined lower values of bee's body weight than the values obtained from feeding with enzymatic inverted sugar syrup, which has proven to be the best source of energy. Eşanu et al,^[59] Analyzing the results obtained from the batches receiving energy-protein recipes, they noticed that the introduction of proteins into the bee's diet first leads to an increase in body weight. Concerning food consumption, they noticed that the highest values of this parameter were recorded in the lots that received only the energy feed. This may be due to the increased attractiveness of these sweet syrups and the easier way of harvesting and processing by bees. Of the energy-protein recipes, the one obtained by blending pollen-powdered with sugar syrup recorded the highest consumption values, while the sugar syrup and yeast mixture recorded the lowest values (low attractiveness). Food consumption did not have a constant evolution or involution because it depended largely on the ingestion capacity of the bees from cages, on the nutrient requirements for the growth of the wax and last but not least on the characteristics of the ingredients used in food preparation, which determined different degrees of filling bee's bowels.

Many studies have demonstrated the great similarity between physiological systems in insects and vertebrates in an unquestionable manner. When comparing the metabolism of sugar and the role of insulin hormone in its regulation and the resulting disorder of dysfunction in similar functions in vertebrates, The probability of disease disorders is large and deserves intensive study to protect hives, ensure the safety of breeds, and the quality of honey produced.^[77]

It has been proposed that one route of behavioral evolution involves novel regulation of conserved genes. Age-related division of labor in honeybee colonies, a highly derived behavioral system, involves the performance of different feeding-related tasks by different groups of individuals. Older bees acquire the colony's food by foraging for nectar and pollen, and the younger "nurse" bees feed larvae processed foods. The transition from hive work to foraging has been shown to

be socially regulated and associated both with decreases in abdominal lipid stores and with increases in brain expression of genes implicated in feeding behavior in *Drosophila melanogaster*. Arrese and Soulagés (2010) showed that division of labor is influenced by a canonical regulator of food intake and energy balance in solitary species, the insulin/insulin-like growth factor signaling (IIS) pathway. Foragers had higher levels of IIS gene expression in the brain and abdomen than did nurses, despite their low lipid stores.^[11]

The fat body plays major roles in the life of insects. It is a dynamic tissue involved in multiple metabolic functions. One of these functions is to store and release energy in response to the energy demands of the insect. Insects store energy reserves in the form of glycogen and triglycerides in the adipocytes, the main fat body cell. Insect adipocytes can store a great amount of lipid reserves as cytoplasmic lipid droplets. Lipid metabolism is essential for growth and reproduction and provides energy needed during extended nonfeeding periods.^[16,98]

Foraging also is affected by experimentally induced changes in the expression of genes related to feeding behavior in *Drosophila*.^[100,50] (iii) Nurses have much larger lipid and protein nutrient stores than foragers.^[11] Large lipid stores may be functionally associated with nursing behavior because bees that are forced to revert from foraging to brood care do not regain large lipid reserves and are not as good at rearing brood as typical nurses.^[112] The striking loss of abdominal lipid that occurs before the onset of foraging,^[11] is thought to increase individual foraging performance,^[25] (iv) Nutritional differences between nurses and foragers occur even though all colony members are exposed to the same food stores inside the hive, further suggesting close coupling of nutritional status and behavior.^[11]

To perform multiple metabolic functions to fulfill the changing physiological needs of the insect during development, the fat body must be able to integrate signals from other organs. Many of these functions are hormonally regulated, and thus the fat body is the target organ of several hormones.^[65,120] At the same time, the fat body responds to the metabolic requirements of the organ itself. Therefore, several metabolic processes in the fat body must be tightly coupled to a number of metabolic pathways.^[11]

Physiological systems to sense nutrient reserves are expected in all organisms, and in insects nutrient sensing itself appears to be the domain of the fat body.^[22] Studies of *Drosophila melanogaster* and, more recently, mosquitoes have shown that the fat body specifically expresses amino acid transporters that function as nutrient sensors.^[18,73] The level of nutrient reserves accumulated in the fat body modulates several important aspects of the insect's life such as the rate of insect growth, the timing of metamorphosis, and egg development.^[69,40] The fat body coordinates insect

growth with metamorphosis or reproduction by storing or releasing components central to these events. For example, the synthesis of vitellogenin in the fat body of *Aedes aegypti* female mosquitoes is transcriptionally upregulated after a blood meal by a mechanism involving a cascade of reactions beginning at the fat body plasma membrane, where specific amino acids present in the hemolymph are sensed by amino acid transporters. This signal activates an evolutionarily conserved nutritional signaling cascade target of rapamycin pathway that- results in the translation of a specific transcriptional activator of vitellogenin gene expression. As a result, the synthesis of vitellogenin is stimulated, reaching a peak 30 h after the blood meal.^[102]

In addition to its role related to storage and utilization of nutrients, the fat body is an endocrine organ,^[76] produces several antimicrobial peptides,^[63] and participates in detoxification of nitrogen metabolism,^[16] Clearly, the fat body is a multifunctional organ, as reflected by the transcriptome pattern observed from *D. melanogaster* and *Bombyx mori*,^[82]

Insulin/insulin-like growth factor signaling (IIS) is a key regulator of both metabolism,^[35] and feeding-related behaviour,^[139] Food intake or high levels of nutrient stores leads to enhanced synthesis of insulin,^[122] or (in insects) insulin-like peptides (ILPs),^[16] and represses the synthesis of glucagon or (the insect equivalent) adipokinetic hormone (AKH),^[58] IIS also up-regulates both the intracellular target of rapamycin (TOR) pathway,^[20] and juvenile hormone (JH),^[21,22] JH is known to be involved in the regulation of honeybee behavioral maturation.^[111,15]

Arrese and Soulagés,^[16] results suggest roles for insulin signaling in the brain and fat body. Increased *ilp1* production in the brain may influence behavior through local action on neuronal circuits that control foraging and also may affect non-brain targets, such as the fat bodies in the abdomen. High levels of *inR1* and *inR2* in the abdomen should maximize the responsiveness of abdominal tissues to circulating ILPs. However, we cannot discern whether the increase in insulin signaling during behavioral maturation is a cause or consequence of lipid loss.

Insulin signaling influences diverse aspects of phenotypic plasticity in honeybees. Insulin signaling has been implicated in the regulation of caste (queen vs. worker) determination in honeybees,^[30,69] and insulin-signaling genes are among the more promising candidate genes located in quantitative trait loci associated with genetic variation for honeybee foraging behaviour.^[70] Several models have been proposed to explain how insulin signaling can influence diverse aspects of phenotypic plasticity in honeybees.^[47,71,70] Arrese and Soulagés,^[16] confirmed a specific prediction of Corona *et al.*^[22] by showing that low nutrient stores can increase insulin signaling. However, the context specificity of this

effect implies that interactions among insulin signaling, nutrition, JH, Vg, and the environment are more complicated than had previously been imagined. Our results support the notion that molecular pathways that govern nutritional state and feeding behavior in solitary animals represent one “toolkit” that can be used in the evolution of division of labor in social insects.^[16] Learning how and why some components of insulin-signaling pathways are more evolutionarily labile than others will help understand the molecular basis of behaviour.^[11]

Whereas many insect tissues have vertebrate analogs, the fat body is an organ unique to insects.^[89] The fat body is a relatively large organ distributed throughout the insect body, preferentially underneath the integument and surrounding the gut and reproductive organ.^[111] Unlike the solid structure of the liver, the insect fat body is a loose tissue. Generally, the organ is arranged in thin lobes that are bathed by the hemolymph. This type of organization provides maximal exposure to the hemolymph. Ready access to hemolymph is vital for the organism to adjust appropriately to the changes in the concentration of energy precursors in circulation. Arrese and Soulages,^[11] This is especially critical under conditions of extreme energy demand such as insect flight, in which the metabolic rate increases 50-to 100-fold.^[132]

All these previous studies and accurate results showed a very similar relationship between the role of insulin in insects and vertebrates, and its importance in physiological activities, growth and developments regulation and molecular biology, which showed the importance of the relationship between hormonal regulation and The raw materials produce energy stored in the fat body and the Similarities of its physiological mechanisms especially in metabolism and detoxification role with liver in mammalian, which leads us in this study to the importance of attention to the type of nutrition provided to the bees, because the impact on all activities of all honeybee body systems furthermore behavior, hormonal regulation and molecular biology, which supports our approach toward the work on specialized studies in this aspect to serve this purpose in different dimensions, due to the complex and accumulate relations with all we mentioned above in terms of productivity, environmental, agricultural, health, and economic. The results of this study contribute to the assessment of the impact of the use of food recipes on some indicators of the quality of bees isolated from colony, but the characterization of the ingredients used in bee nourishment is still an interesting subject for practitioners and researchers in this field.

Conclusions and future prospects: Honey possesses numerous biological, biochemical and physiological activities in animals, bees as well as in humans. The efficacy of these properties depends on the compounds present in the honey. Two different types of honey have

been investigated to compare their effect on blood glucose, insulin and obesity. The results provided in this study makes clear the need for evaluation of the many potential biological and pharmacological activities of honey with Pepsi source, including in the treatment of diabetes and metabolic disorders.

Bees belong to the animal organisms according to text of the insect physiology,^[87] there for it shares the same physiological cellular processes such as metabolism, with other living organisms, permanently feeding bees on a soda drink causes damage to the health of the colonies and producing a product containing the ingredients that may affect consumers health, efficiency and productivity of community members. Unhealthy nutrition is an international phenomenon, widespread around the world leading to the prevalence of chronic diseases among the people. Chronic diseases contribute in raising the health care budget for countries.

The Honey production companies have no sympathy with customers. Their first and only goal is to increase their sales, no matter if it must cost human lives. People are being widely affected by the soft drinks on a large bases and teething problems, obesity, diabetes, Alzheimer and fatty liver disease became common now. Results of this study, indicates that the honey derived from the bees feed on the Pepsi, consists from its contents necessarily, may lead to the sugar disorders diseases, in addition to the damage of colonies of bees, through the high mortality which were recorded compared to the colonies which fed on natural nectar.

Honey has the potential to be the vector of a range of microbial diseases including American foulbrood, European foulbrood, chalkbrood, and nosema disease. American foulbrood disease is fatal to a bee colony. The others are considered serious economic diseases that have the potential, in some cases, to kill the colony. In the case of beekeepers continue to feed the bees on the Pepsi, maybe lead to health problems and weakness of immunity system which is assumed by the results of this study.

According to the current study the Saudi honey with complete natural sources close to the International Honey Quality Standards it showed long shelf life due to natural moisture contents. Two quality parameters i.e. diastase number and moisture content are quite distinctive and can be used as parameters to identified honey based on bee species type. It appears that International Honey Standards are based on honey produced by *A. mellifera*, fit with standard honey composition of Asian species.

Suggestions for further research: Research context was limited about feeding bees on soft drink in sector of honey producing farms in Saudi Arabia, while the future research can be extended to different sectors of agriculture sub sector across the country get more significant results. There is also needed to conduct a

research on the possibility of value addition for the honey harvested by farmers to ensure that they get more value for their honey.

There are a range of sugar products available to bee feed, there for State institutions concerned in quality in the production of honey should contribute to answer the main concern for beekeepers is what effect Pepsi supplements have on the colony, by support the studies concern with this solving this problem, which guide the beekeepers to the ideal food for colonies requiring supplementation.

RECOMMENDATIONS

1. It is essential that the government and relevant development partners work hand in hand to design and promote forums through which bee farmers can be trained on management of beehives, identification of hive products and how to add value onto the products.
2. The Ministry of Agriculture and Trade monitor the production of honey and ensure its quality to, ensure the marketing honey from natural sources only, prevent derivative species on soft drinks.
3. The central government in conjunction with the county government should carry out public education on how to improve bee keeping and the quality and quantity of honey they produce.
4. Farmers should also be educated on where to access services and advice given the new system of devolved governance.
5. Financial education is also very necessary for the farmers since many farmers do not keep records of the income, they get from selling honey.
6. Financial empowerment is necessary to ensure that farmers improve the quality and quantity of honey.
7. The financial help will help farmers improve their harvesting, processing and storing methods.
8. Financial help too will farmers' accessibility to markets, far beyond the county level thereby increasing income from hive products.
9. Endocrinologists, dietitians and diabetes therapists should educate the patients and their families in nutritionist and weight loss programs, need to mention the importance of the source of honey, and emphasis on the selection of honey derived from sources natural nectar , to ensure the quality of the honey before it is included in the diet
10. further research on the possibility of incorporating natural honey from natural sources extracted from nectar into weight loss programs, control of sugar level in blood

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