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Original Research Article

The Effect of Various Carbohydrate Sources On Half-Life, Biological Traits and Microbial Flora of the Bee Digestive System

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ABSTRACT

The research was conducted in a completely randomized design in 12 treatments and 6 replicates in an incubator on Apis Mellifera Meda, which started from the first half of May 2018 and was carried out until the end of July at the Isfahan Agricultural Research Center. The treatments included white sugar, brown sugar, honey as control and corn liquid fructose, corn starch, wheat starch and potato starch in sweet dough, which was tested in proportion of 15% and 30%. The measured traits consisted of feed intake, half-life, carcass protein and lactobacillus and Coliform content of honey bees' digestive systems. The results indicated that the treatments showed a significant difference in all traits ($p < 0.05$) and the highest feed intake was related to honey treatment (42.50 g) and the lowest amount of sweet dough containing 30% wheat starch (21.50g), the lowest 50% mortality rate was related to sweet dough (40 days) and the highest mortality rate was related to sweet dough containing 30% corn starch (10 days) .According to the results, sweet dough treatments containing 15% starch in total showed better results than sweet dough treatments containing 30% starch.

Keywords: Incubator, Half Life, Coli bacillus, Coliform, Feed intake

INTRODUCTION

Researchers, scientists and severely beekeepers have a significant consideration of nutrition of honeybees to solve the upcoming challenges that affect their ability to stay healthy and improve the efficiency of production (Keller et al. 2005; Toth et al. 2005; Alaux et al. 2010). This condition gets intense in commercial bee operations that include a diverse management style regarding the colonies movement, quality and amounts of food. An intelligent decision on how to keep a honeybee is possible only when the fundamental demands for feeding bees correctly and in perfect detail has been investigated. In these insects, nutrition should be considered in a completely two independent manners, since the larval period is usually different from the adult insects. However, the process of feed intake of larva and adult bee are relatively close since a matured insect should actively and gradually feed larvae (Hrassnigg and Crailsheim 2005). Nutrition involves all the operations by which an organism converts various nutrients, minerals, water, vitamins and other substances into body parts or acquired energy for various vital processes (Hrassnigg and Crailsheim 2005).

Pollen, produced by flowers, is the major source of protein for honeybees (Roulston and Cane 1999) though it does not provide energy for the bee. Pollen grain supplies all the requirements of the colonies in terms of protein that plays a vital role in the growth of the body and is essential for the restoration of tissues and other body functions. The bulk of the contents of the pollen and honey is made up of glucose, fructose and sucrose, while the additional sugars content found in nectar, have less nutritive value. Considering the incapability of honeybees to break the additional content down, the utilized percentage has toxic effect during the ingestion (Johansson and Johansson.1976). In an emergency sugar and carbohydrates sources feeding can be used as a supplementary material or substitutes (Hendriksma and Shafir 2016). when the colony is running short of stored honey, especially in winters. For this purpose, dense phase of material is most probably suitable for feeding to bees. Attention to the protein components of the diet has to be taken into account to increase the population numbers.

The economic development of the maintenance of the honey bee directly goes back to the health of bee colonies. Several effective bacterial pathogens have been identified on honeybee hives including the American Luke and European Luke disease. Kelly Bacillus larvae is the cause of the American Luke disease in infants and adult honey bees, but so far little studies have been done on the microflora in the honeybee hives. It has often been reported that most of the bacteria in bees' comb and adult bees belong to Bacillus genus (Gilliam et al.1990; Stangaciu.1997) Most of the bacteria isolated from the stomach and intestines of the bee colonies belonged to the same groups that were separated from the body. Sporadic bacteria were dominant in the stomach, while spores and

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lactobacilli excreted in the intestine, and the aerobic spore bacilli dominated the bacteria on the surface of the body (67-83% of microcucci and Sarsis 20-8%, While gram negative bacteria, actinomyceses and lactobacilli were less than that). Microorganisms isolated from the body surface of the bees include Bacillus subtilis, Cereus, Budius, Pomilus, Coagulans, Megatrium, Sarcas, Microcokes, Lactobacillus, Streptomyceses, Candida and Saccharomyces, in addition to Coliforms and other germs of the Gram-negative ones have also been identified. It is interesting to note that the same micro fluoric groups and even the same species and genus that have been isolated from the surface of the worker bees have also existed in the stomach and intestines of the bees (Rosa et al.2003; Menezes et al. 2013).The dominant sporadic bacteria present on the surface of the bees, stomach, and intestines indicate that the common bacteria present on the flowers that are visited by the bee are consistently constant in the digestive system, since nectar and pollen are the only source of bee feed. In Intestines, sporadic bacteria are not only dominant, but also lactobacilli species are significant. This condition is probably due to the ideal conditions in the intestines for these bacteria. In the case of intestinal bacterial populations, sporadic bacteria are 46-41%, lactobacilli are 30-33%, and gram-negative bacteria contain 17% of bacteria. Streptococci and micrococci have been reported to a lesser extent. In the case of gastric bacteria, dominant sporadic basil is constituted of isolated bacteria and contain 61.6%, lactobacilli and streptococci were 31%, while micrococci, antinomies and gram-negative bacteria were not always present. All isolated yeast in the stomach are limited to two species found on the body, including Candida (63%) and Sacharomycesis (37%). In general, the determinants of the microorganisms' types are the honey and digestive system of the bees, and nutrition is a source of influence on the microbial flora (El-Leithy and El-Sibaei. 1972; Yoshihama and Kimura. 2009) It has also been shown that bacteria derived from neonate cells are less than the number of microflora of adult bees. In addition, it has been observed that the cultured and derived bacteria from neonatal samples increase from winter to summer, this issue is likely to return to colonial activities during these seasons, and most of these bacterial groups belong to Basil Coronobacterium. The observation of several microorganisms, such as those of Bacillus genus (In principle, those who have biological control abilities) and the differences observed between infected bacteria and microbial flora of adult bees have led researchers to be encouraged to explore more about the microbiology of honeybee and new strategies for controlling Pathogens (El-Leithy and El-sibael.1992; Piccini et al. 2004).

The aim of this study was to investigate the sources and levels of carbohydrates in feeding honey bee and replacing these sources with white sugar to enhance performance, increase the quality of honey as well as affordable prices, ease of supply and usability for honeybees.

MATERIALS AND METHODS

Treatment and Experimental design

In this research, the effect of some carbohydrate sources on the biological traits and microbial flora of the honey bee in incubator was examined. In this experiment, 12 treatments and 4 replications were started in the form of a completely randomized design of Apis Mellifera Meda from the second half of May, 2018 and the second half of July was carried out and the location of the project was conducted at Isfahan Agricultural and Natural Resources Research Center. The tested treatments included control treatment (honey, white sugar and brown sugar), sweet dough containing (25% honey and 75% white sugar), sweet dough containing 15% potato starch, sweet dough Containing 30% potato starch, sweet dough containing 15% wheat starch, sweet dough containing 30% wheat starch, sweet dough containing 15% corn starch, sweet dough containing 30% corn starch, sweet dough containing 15% corn liquid fructose and sweet dough Containing 30% corn liquid fructose. The measured traits in the incubator include feed intake, protein content, fat and carcass dry matter, 50% mortality (half-life), and microbial flora of the digestive system (Lactobacillus and Coliforms).

One-day bees for use in incubator

To test and minimize mistakes and to achieve maximum success in terms of longevity, newborn bees were used, and because all the tested bees were workers, there was no sex difference. For this purpose, inside a metal shelf, enclosed by a barrier network, the queen was enclosed and cleaned up comb, and the queen was hanged on comb and inside the cage. In this way, the queen did not have the power to get out of the cage and was forced to lay on comb but the worker bees could easily enter and exit the queue for nourishment and nursing. On the basis of this framework, the date on which the Queen was enclosed was recorded. Twenty-four hours later, it was removed from the cage, and in order to breed, the eggs were placed in the middle of the hive and adjacent to the cage, and

then another new one replaced their previous one, and the same operation was repeated and for counting one-day bees and transferring them to incubator cages.

Method of counting one-day bees for transferring them to incubator cages

The method (Gary and Lorenzen.1988) was used, which is as follows. On the floor of a plastic bucket, a moonlight bulb ring was embedded and at the top of the bucket, a neon grip is placed to collect the moonlight and as a result, the bees were drawn to the center of light. This grip covers the bucket and, in order to prevent the bees from getting out of the wall of the bucket, the wall of bucket between the grip and the upper edge is impregnated with Vaseline oil. To count the one-day bees, the frames containing bees were transferred to a dark room on this neon grip so, the bees were stopped due to the light-induced nature of the neon surface, which made counting operations easy. This method has no negative effect on the bees. Fluorescent lights preferred to ordinary light because it does not generate too much heat. To get the bees from the surface of the page, the bucket was used from a small manual vacuum pump that looked like a vacuum cleaner. After the number of bees counted to two hundred, the tank was separated from the vacuum pump and transferred to the cage by a funnel on the cage's head. In conducting microbiological experiments, in order to investigate the microbial flora changes of the digestive system of the adult bees, after reaching the bee mortality to 50% (half-life).

Method of measurement of the microbial flora of the digestive tract

The remaining bees were transferred to the laboratory for microbiological testing and the bees were placed immediately in the freezer at a temperature of -18° C to minimize their activity and immediately removed the whole digestive system of the honey bees for microbiological testing. To dilute the isolated digestive system, the serum of physiology was used. To this end, 5.5 g of NaCl were dissolved in one liter of distilled water. Then they poured 9 cc into the test tubes, closed their doors with cotton and then autoclaved. One gram of fecal specimen was poured into the tubes and shake to obtain a dilution of 0.1. With the aid of a sampler, 1000 µl of the prepared sample was poured into the second tube and shake until a dilution of 0.01 was obtained. From this dilution, 1000 µl was transferred to the third tube and shake to achieve a dilution of 0.001, and the dilutions of 0.0001, 0.00001, 0.000001 in the fourth, fifth, sixth and seventh tubes were prepared. Then, from solution 5, 4, 3, and 6, the tubes were inoculated on MacConkey culture media for culture of Coliform and on MRS culture media inoculated with 200 ml of lactobacillus in each plate. It was then sprayed uniformly with a flat glass tube. The Coliform culture media prepared inside the incubator and MRS culture media was packed in an anaerobic liner and closed tightly in the jars. All culture media were transferred to a 37 ° C in incubator. The Coliform population was counted after 24 hours and the count of lactobacilli was performed after 72 h (Tajabadi et al. 2011, 2014; Sarbojoy. 2018).

Calculation of carcass protein

To Calculation of carcass protein content, because the bees keep their stool in the rectum and do not repel, rectum and total intestine were removed by pence so that no feces remain in the intestines. After that, the sample was transferred to the laboratory to calculate the protein, fat and dry matter of the carcass. Kjeldal method was used to determine the protein content of the samples, (Graffin and Horwitz. 1988).

Calculation of carcass fat

Calculation of carcass fat (carious ether extract) was performed by Soxhlet and Diethyl ether solution for 6 hours. Other actions related to the extraction of fat from samples were made in accordance with the suggestion and guide of the device.

Calculation of the amount of dry matter of carcasses

To Calculate the amount of dry matter of carcasses, dry oven was used at 105 ° C. After drying, the samples were transferred to the Desiccator and weighed after cooling. The weight of the specimens was measured with accuracy before and after drying using a scale of 0.001. Every day at 10 o'clock, dead bees were removed from the little hives and were counted. Daily, until the end of the experiment, the feed intake was measured with a precision of 0.1 g.

Incubator

A chamber with dimensions of 1.8 × 1.8 × 2.5 m is insulated by thick aluminum sheet. The chamber was equipped with a 30×30 cm fan and an 800-watt electric element fitted with a thermostat. The incubator temperature was kept constant at 34 ° C. The room humidity is adjusted to about 60% relative humidity, which is provided by wet sacks in the bottom of the chamber, as well as by the bottom of the chimney, and the moisture content is measured by a humidifier. A thermometer was installed to record the temperature. The room was connected to an additional door with free space to minimize the thermal stresses and air flow inside the room.

Statistical analysis

Derived data were recorded by excel software and then analyzed for variance analysis using SAS (Statistical Analysis System) software which is developed by(SAS 9.0 Institute .2000) for advanced analytics applying GLM procedure. Applying Duncan's multiple range tests, the average was compared at a probability level of 5%. All of parameters were examined as follows:

$Yij = \mu + Ti + eij$

Where Yij is individual observation, μ is the overall mean, Ti is the effect of treatment, and eij shows the random error.

RESULTS AND DISCUSSION

Feed intake

In the research, the mean of feed intake among treatments was significant at a level less than 5%. According to the results of dietary intake during incubator period (40 days), the highest amount was consumed for honey treatment (42.5 g) and the lowest amount was for sweet dough containing 30% wheat starch, which difference was less than 5 The percentage was significant ($p < 0.05$) (Table 1). Between sweet dough treatments containing starch, sweet dough containing 15% corn starch showed a significant difference with the rest of sweet dough containing starch ($p < 0.05$). In total, sweet dough containing 15% starch in comparison to sweet dough contain 30% higher starch consumption, which is consistent with (Abadi et al. 2018) results. The amount of white sugar after honey treatment is higher in comparison to the rest of the treatments, which is consistent with the results of (Ruiz-Matute et al. 2010), which indicates that the amount of white sugar is higher than the rest of the treatments. (Vogel. 2000) reported that the total amount of sugars consumed by sucrose bee is most relevant to the acceptance and nutritional value of bees, which is consistent with the results of the research. (Barker and Lehner. 1978) reported that the corn syrup containing high fructose syrup was well consumed in terms of feed intake, which is consistent with the results of this study. (Behjatian et al. 2006) reported that feed intake to the bee population depends on an increase in the mortality rate of feed intake, which was found in the research. Sweet dough treatment with the lowest casualties did not have the highest feed intake. Therefore, it seems that the consumption of food in addition to the bee population is also related to the sweetness of the food pulp.

Table 1. The effect of different sources of carbohydrates on the average amount of feed intake .values are means ±SD.

Controlling Treatments		Average of Feeding rate (g)
Honey		$42.50^{a} \pm 1.73$
White sugar		$40.75^{a} \pm 0.957$
Brown sugar		$41.50^{a} \pm 1$
Sweet dough containing (%)	SD (25 honey & 75 white sugar)	$26.5^{bc} \pm 1$
	SD (15 potato starch)	$25^{cd} \pm 1.41$
	SD (30 potato starch)	$23.5^{d} \pm 0.577$
	SD (15 wheat starch)	$25^{cd} \pm 1.15$
	SD (30 wheat starch)	$21.5^{e} \pm 0.577$
	SD (15 corn starch)	$27^{b} \pm 1.15$
	SD (30 corn starch)	$21.75^{e} \pm 1.5$
	SD (15 corn liquide fructose)	$25.25^{bcd} \pm 1.3$
	SD (30 corn liquide fructose)	$25.25^{bcd} \pm 0.957$
(P-value)		P <0.0001
SEM		3.55

SEM: standard error of means. Footnotes (a-e) show significant differences each column ($p<0.05$).

Mortality (half-life)

The average time of 50% mortality (half-life) of honey bees was significant at 5% level among treatments ($p < 0.05$). Sweet dough containing (25% honey and 75% white sugar) (40 days) showed a significant difference with other treatments ($p < 0.05$) that The highest mortality rate was observed for 15% corn starch (11.50 days) and sweet dough containing 30% corn starch, which reached 50% mortality for (10 days) (Table 2). After serving sweet dough containing (25% honey and 75% white sugar), sweet dough containing 15% potato starch was sweet dough containing 30% corn liquid fructose and sweet dough containing 15% corn liquid fructose in the next place, respectively that this study was conducted with(LeBlanc et al. 2009) that fructose has no toxic effect on the bee and increases the population. (Burenside et al. 2000) reported that the amount of sugars consumed with the lifetime of the bees, but it was not clear that accepting low sugar is a major cause of mortality or the toxicity of some sugars. There was no significant difference between white sugar and brown sugar in control treatments, but there was a significant difference with honey ($p < 0.05$). Sweet dough containing 15% starch had a lower mortality rate than sweet dough containing 30% starch, which is consistent with (Abadi et al. 2018) the results, that states sweet dough containing 15% starches increase the population.

Table 2. The effect of various carbohydrate sources on the average mortality (half-life). values are means ±SD.

Controlling Treatments		Honey bees' half-life (day)
Honey		$13^{gf} \pm 0.816$
White sugar		$20^{d} \pm 1.41$
Brown sugar		$20^{d} \pm 0.816$
Sweet dough containing (%)	SD (25 honey & 75 white sugar)	$40^{a} \pm 0.816$
	SD (15 potato starch)	$37^{b} \pm 0.816$
	SD (30 potato starch)	$19.5^{d} \pm 1.29$
	SD (15 wheat starch)	$16.5^{e} \pm 0.577$
	SD (30 wheat starch)	$13.5^{f} \pm 1.29$
	SD (15 corn starch)	$11.5^{gh} \pm 1.29$
	SD (30 corn starch)	$10^{h} \pm 1.41$
	SD (15 corn liquide fructose)	$32.5^{c} \pm 1.29$
	SD (30 corn liquide fructose)	$36.75^{b} \pm 0.957$
(P-value)		P <0.0001
SEM		5.33

SEM: standard error of means. Footnotes (a-h) show significant differences each column ($p<0.05$).

Carcass protein

The highest mean of carcass protein was found in sweet dough treatments containing 30% corn liquid fructose (23.5%) and sweet dough containing 15% corn starch (23.4%) which did not show any significant difference ($p > 0.05$). Table 3 but with other treatments the difference was significant and the lowest carcass protein was related to honey (21.50%) that these results were consistent with the results of (Jasmien et al. 2014) that corn starch, wheat and potatoes had the highest corn starch content, indicating that the consumption of sweet dough containing corn starch saved the highest amount of protein in the carcass (Brodschneider and Crailsheim. 2010). reported that the newborn bee colony protein content was 13% and the 5-day bee was 15.5%. (Cran. 1990) also reported that the raw protein content of bee hull is 49.8%, based on dry matter. In the control treatments, no significant difference was observed between white sugar and brown sugar treatments ($p < 0.05$). But honey treatment with white sugar was significantly different. Among the sweet dough containing starch, the highest amount of proteins related to sweet dough treatments containing 15% corn starch showed a significant difference with other treatments of sweet dough containing starch ($p < 0.05$).

Table 3. The effect of various carbohydrate sources on the average carcass protein. values are means ±SD.

Controlling Treatments		Average carcass protein (%)
Honey		$21.5^{h} \pm 0.081$
White sugar		$21.7^{g} \pm 0.141$
Brown sugar		$21.6^{gh} \pm 0.081$
Sweet dough containing (%)	SD (25 honey & 75 white sugar)	$21.5^{h} \pm 0.081$
	SD (15 potato starch)	$22.1^{f} \pm 0.014$
	SD (30 potato starch)	$22.5^{d} \pm 0.141$
	SD (15 wheat starch)	$22.38^{ed} \pm 0.008$
	SD (30 wheat starch)	$22.3^{e} \pm 0.008$
	SD (15 corn starch)	$23.4^{a} \pm 0.141$
	SD (30 corn starch)	$22.97^{b} \pm 0.016$
	SD (15 corn liquide fructose)	$22.8^{c} \pm 0.081$
	SD (30 corn liquide fructose)	$23.5^{a} \pm 0.081$
(P-value)		P <0.001
SEM		0.345

SEM: standard error of means. Footnotes (a-h) show significant differences each column ($p<0.05$).

Carcass fat

The highest mean of carcass fat was found in sweet dough treatments containing 15% wheat starch (4.42%) and the lowest amount of sweet dough containing 30% potato starch (3.63%), which showed a significant difference ($p <0.05$) Table 4.(Jasmin et al. 2014) reported that between corn starch, wheat starch and potatoes starch, lipids, and glycolipid are more common in wheat starch treatments than any other which was consistent with the results of this study, which showed that the amount of fat in carcass was increased in treatments using wheat starch containing sweet dough. (Cran. 1990) reported that the fat content of larvae was 2.39%, and the pupae was 3.71%. The researcher also stated that the glycogen content of mature larvae was 41 percent and puppies was 0.75 percent. (Roulston. 2004) reported that high and densely populated colonies are less vulnerable to pests and pathogens and will easily leave cold and long winters due to the storage of carbohydrate fat. With the timely supply of protein and lipophilic requirements, the pharyngeal glands, the wax glands and the toxin glands of the worker bees are activated and provide the necessary food for the development of the larvae and as a result, the queen becomes more egg-laying, and the population gradually increases. (Haydak. 2000) reported that as the age goes up, the magnitude of the head and neck of the queens and male increases, and peaked in periods of life of this form.

Table 4. The effect of various carbohydrate sources on the average carcass fat .values are means ±SD.

Controlling Treatments		Average carcass fat (%)
Honey		$3.95^{c} \pm 0.021$
White sugar		$3.80^{g} \pm 0.008$
Brown sugar		$3.88^{e} \pm 0.021$
Sweet dough containing (%)	SD (25 honey & 75 white sugar)	$3.89^{de} \pm 0.021$
	SD (15 potato starch)	$3.85^{f} \pm 0.021$
	SD (30 potato starch)	$3.63^{j} \pm 0.021$
	SD (15 wheat starch)	$4.42^{a} \pm 0.016$
	SD (30 wheat starch)	$4.35^{b} \pm 0.021$
	SD (15 corn starch)	$3.75^{h} \pm 0.021$
	SD (30 corn starch)	$3.80^{g} \pm 0.021$
	SD (15 corn liquide fructose)	$3.72^{I} \pm 0.021$
	SD (30 corn liquide fructose)	$3.91^{d} \pm 0.012$
(P-value)		P <0.001
SEM		0.115

SEM: standard error of means. Footnotes (a-j) show significant differences each column ($p<0.05$).

Carcass dry matter

In the study, the highest amount of dry matter belonged to sweet dough containing 15% corn starch (33.4%) and the lowest amount of dry matter related to brown sugar treatment (32.20%), which showed significant difference ($p <0.05$) Table 5. (Cran.1990) reported that the dry matter content of larvae was 23% and dry matter of pupae was 29.8%. They also stated that the percentage of dry adult bees was 33.5%, and stated that whatever diet is

balanced in terms of protein and other nutrients, the percentage of dry matter of bee honey is higher, which is consistent with the results of the research. In this study, the amount of sweet dough containing 15% corn starch and sweet dough containing 30% fructose in corn showed the highest amount of carcass proteins, which also increased dry matter content. (Detroy and Harp.1976) reported that the food (ration) in terms of protein, vitamins, minerals and other nutrients would be appropriate to the needs of the bee and would increase the carcass dry matter, which is consistent with the results of this research. There was no significant difference between control treatments (white sugar, brown sugar and honey).

Table 5. The effect of various carbohydrate sources on the average carcass dry matter. values are means ±SD.

Controlling Treatments		Average carcass dry matter (%)
Honey		$32.4^{de} \pm 0.081$
White sugar		$32.5^{de} \pm 0.081$
Brown sugar		$32.2^{e} \pm 0.081$
Sweet dough containing (%)	SD (25 honey & 75 white sugar)	$32.6^{cde} \pm 0.081$
	SD (15 potato starch)	$32.7^{cd} \pm 0.141$
	SD (30 potato starch)	$32.8^{cd} \pm 0.081$
	SD (15 wheat starch)	$33^{bc} \pm 0.081$
	SD (30 wheat starch)	$32.6^{cde} \pm 0.081$
	SD (15 corn starch)	$33.4^{a} \pm 0.081$
	SD (30 corn starch)	$32.6^{cde} \pm 0.141$
	SD (15 corn liquide fructose)	$32.4^{de} \pm 0.081$
	SD (30 corn liquide fructose)	$33.3^{ab} \pm 0.141$
(P-value)		P <0.001
SEM		0.205

SEM: standard error of means. Footnotes (a-e) show significant differences each column (p<0.05).

Amount of lactobacillus

The highest level of lactobacillus was related to sweet dough containing 15% wheat starch (15.09 log CFU/g), and the lowest amount of sweet dough containing 15% potato starch (13.81 log CFU/g) which Showed a significant difference at 5% level (p <0.05) Table 6. Overall, the level of lactobacilli was higher in comparison with the digestive system coliforms, which is consistent with the results of (Gilliam.1998), which reported that lactobacillus is the most common microorganism in the digestive system of honey bees. Bacillus in the honey bee produces some enzyme from the antibacterial material itself. A study on bacillus-containing food sources also suggests that these bacteria act with the help of the chemical process to improve and preserve colony-stored food and produce the compounds needed for pre-digestion (Gilliam. 1997) There were no significant differences among control treatments at 5% level (p <0.05).

Table 6. Effect of various carbohydrate sources on the average amount of produced lactobacillus in the digestive system of honey bees. values are means ±SD.

Controlling Treatments		The amount of lactobacillus produced in the digestive system (log CFU/g)
Honey		$14.5^{c} \pm 0.154$
White sugar		$14.55^{c} \pm 0.100$
Brown sugar		$14.51^{c} \pm 0.110$
Sweet dough containing (%)	SD (25 honey & 75 white sugar)	$14.52^{c} \pm 0.109$
	SD (15 potato starch)	$13.81^{f} \pm 0.074$
	SD (30 potato starch)	$14.52^{c} \pm 0.147$
	SD (15 wheat starch)	$15.09^{a} \pm 0.081$
	SD (30 wheat starch)	$14.24^{d} \pm 0.120$
	SD (15 corn starch)	$13.9^{ef} \pm 0.130$
	SD (30 corn starch)	$14.91^{b} \pm 0.098$
	SD (15 corn liquide fructose)	$14.24^{d} \pm 0.114$
	SD (30 corn liquide fructose)	$14.02^{e} \pm 0.156$
(P-value)		P <0.001
SEM		0.19

SEM: standard error of means. Footnotes (a-f) show significant differences each column (p<0.05).

Amount of coliform

In this study, the amount of coliform in the gastrointestinal tract of honeybee in sweet dough containing 30% corn starch (13.45 log CFU/g) was highest and lowest in sweet dough (11.50 log CFU/g) which showed a significant difference Table 7. In sweet starchy treatments, sweet dough containing 15% corn starch and sweet dough containing 15% wheat starch had the highest level of Coliform (13.30 log CFU/g) which did not show any significant difference, but showed a significant difference with other treatments. Increasing lactobacillus and reducing the bacteria of the coliforms are effective on the length of the lice and the depth of the intestinal crypts and increases the digestibility of the digestive system (Khojasteh and Shivazad. 2006) Also, (Khojasteh and Shivazad. 2006) reported that pathogens such as chlorophylls producing phospholipase A2 can reduce appetite, which is consistent with the research, which contains 30% corn starch with the highest coliform in the digestive system and the lowest amount of food intake.

Table 7. The effect of different sources of carbohydrates on the average amount of produced Coliform in the digestive system of honey bees. values are means ±SD.

Controlling Treatments		The amount of Coliform produced in the digestive system (log CFU/g)
Honey		$12.95^{b} \pm 0.051$
White sugar		$13.01^{b} \pm 0.081$
Brown sugar		$12.96^{b} \pm 0.069$
Sweet dough containing (%)	SD (25 honey & 75 white sugar)	$11.5^{d} \pm 0.154$
	SD (15 potato starch)	$11.59^{d} \pm 0.188$
	SD (30 potato starch)	$12.89^{b} \pm 0.075$
	SD (15 wheat starch)	$13.3^{a} \pm 0.087$
	SD (30 wheat starch)	$12.2^{c} \pm 0.105$
	SD (15 corn starch)	$13.3^{a} \pm 0.040$
	SD (30 corn starch)	$13.45^{a} \pm 0.106$
	SD (15 corn liquide fructose)	$12.2^{c} \pm 0.072$
	SD (30 corn liquide fructose)	$11.6^{d} \pm 0.130$
(P-value)		P <0.001
SEM		0.36

SEM: standard error of means. Footnotes (a-d) show significant differences each column, ($p<0.05$).

Lactobacillus ratio to produced Coliform

In this study, the relationship between lactobacillus on coliforms was found to be highest for sweet dough containing (25% honey and 75% white sugar) treatments with a ratio of (1.26 log CFU/g) and the lowest amount for sweet dough containing 15% corn starch with ratio of (1.04 log CFU/g) that showed a significant difference in the level of less than 5% ($p <0.05$) Table 8.

It was observed in this study that as the ratio of lactobacilli to coliforms is higher, the mortality rate decreases. In the case of sweet dough which had the highest ratio of lactobacillus to the coliform, there was a lower level of mortality and in the sweet dough containing 30% and 15% corn starch with the highest mortality rates, the lowest ratio of lactobacillus to coliform was observed. Which is consistent with the results of (Schrezenmeir and Devrese. 2001), which lactobacilli bacteria in the digestive system are beneficial for honey bee and It seems that any mechanism that can affect the digestive system of the honey bee that increases the population of lactobacillus bacteria is useful for the health and performance of the honeybee. It seems that one of the reasons for the decrease in bees in the testicles is toxicity due to the accumulation of these substances and the maintenance of stool in the body for a long time and any mechanism that can affect the digestive system of the bee in a way that increases the population of lactobacillus is beneficial for the health and performance of the honeybee (Dalloul et al.2003).

Table 8. The effect of various carbohydrate sources on the average ratio of lactobacillus to produced Coliform of the digestive system of honey bees . values are means ±SD.

Controlling Treatments		Lactobacillus ratio to produced Coliform of the honey bee digestive system (log CFU/g)
Honey		$1.11^{de} \pm 0.008$
White sugar		$1.11^{de} \pm 0.005$
Brown sugar		$1.11^{de} \pm 0.002$
Sweet dough containing (%)	SD (25 honey & 75 white sugar)	$1.26^{a} \pm 0.009$
	SD (15 potato starch)	$1.19^{b} \pm 0.019$
	SD (30 potato starch)	$1.12^{de} \pm 0.005$
	SD (15 wheat starch)	$1.13^{d} \pm 0.002$
	SD (30 wheat starch)	$1.16^{c} \pm 0.019$
	SD (15 corn starch)	$1.04^{f} \pm 0.006$
	SD (30 corn starch)	$1.1^{e} \pm 0.001$
	SD (15 corn liquide fructose)	$1.16^{c} \pm 0.016$
	SD (30 corn liquide fructose)	$1.2^{b} \pm 0.026$
(P-value)P <0.001		
SEM0.03		

SEM: standard error of means. Footnotes (a-f) show significant differences each column ($p<0.05$).

CONCLUSIONS

The nutritional effects of a food must be tested in different ways. Only accepting and consuming that substance by honey bees cannot be a precise criterion for judging its quality. In addition to its intake, other factors such as the effect of that substance on colony population, brood population and production honey should be investigated. starch sources can be used in feeding bees, but the research showed that due to the different starch structure, the effect of each starch source on the performance of honey bees is different, and the level of 15% of starch in the total yield of honey bees compared to Level increased by 30%. In this research, other studies, such as heating, and the amount and duration of heating of various starch sources, can be studied, and their impact on consumption and performance increase.

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REFERENCES

Abadi H.R.K.S., Shahryar H.A., Shaddel Teli A.A. and Nobar S. (2018). Impact of various carbohydrate sources on functional attributes, colony population, feed intake, and quality of honey produced by the honeybee. Journal of Kafkas Univ Vet Fak Derg, 24 (4): 503-510.

Alaux, C., Ducloz, F., Crauser, D. and Conte, Y. L. (2010). Diet Effects On Honeybee Immune competence. Journal of Biology Letters, 6(4),562-56.

Barker R.J. and Lehner Y. (1978). Laboratory comparison of high fructose corn syrup, grape syrup, honey, and sucrose syrup as maintenance food for caged honey bees.

Behjatian Esfahani M., Qaysari A., Jalali Zand A., Abbasian A. and Nilfrooshan A. (2010). The nutritional effects of different levels of probiotic on longevity and chemical composition of honey bee. Fourth Iranian Congress of Animal Sciences.

Brodschneider R. and Crailsheim K. (2010). Nutrition and health in honey bees. Journal of Apidology, 41 (3): 278–294.

Burenside C.E.and Vvansell G.H. (2000). Plant poisoning of bee. use Agrico. Plant quarantine mimeo, 39.12.10.

Cran E. (1990). Bees and beekeeping. British Library cataloguing in publication date 469- 470.

Chapman R.F. (1982). The Insects: Structure and Function. Harvard University Press.

Dalloul R.A., Lillehoj H.S., Shellem T.A. and Doerr J.A. (2003). Enhanced mucosal immunity against Eimeria acervulion in broilers fed a Lactobacillus-Based probiotic

Detroy B.F. and Harp E.R. (1976). Trapping pollen from honey bee colonies. American bee journal.

El-Leithy M.A. and El-Sibaei K.B. (1972). External and internal microflora of the honey bee (A.mellifera L.). Egyptian Journal of Microbiology.7: 79-87.

El-leithy M.A. and El-sibael K.B. (1992). Role of microorganisms isolated from bees, its ripening and fermentation of honey. Egyptian Journal of Microbiology. 75:679–681.

Gary N. E. and Lorenzen K. (1988). Substage Illumination device for restraining flight of honey bees (Hymenoptera: Apidae) and positively. Phototactic Insects, 7:79-87.

Gilliam M., Roubik D.W. and Lorenz BJ. (1990). Microorganisms associated with pollen, honey, and brood provisions in the nest of a stingless bee. Melipona fasciata. Apidologie . 21: 89-97.

Gilliam M. (1997). Identification and roles of nonpathogenic associated with honey bee. Federation of European Microbiological Microbiology letters.

Graffin A. and Horwitz C. (1988). Determination of Protein Content by Kjeldahl Method. Journal of AOAC. vol.71, nr.5:893-897

Haydak M.H. (2000). Brood rearing by honey bees confined to a pure carbohydrate diet Journal of Economic Entomology 28:657-600.

Hendriksma H.P. and Shafir S. (2016). Honey Bee Foragers Balance Colony Nutritional Deficiencies. Journal of Behavioral Ecology and Sociobiology 70(4), 509–517.

Hrassnigg N. and Crailsheim K. (2005). Differences in drone and worker Physiology in honeybees (A.mellifera). Journal of Apidology. 36(2), 255–277.

Johansson TSK.and Johansson MP.(1976). Feeding sugar to bees. Bee World 57: 137-143; 58(1): 11-18; 58(2): 49-52.

Jasmien W., Sara V ., Ellen F and Jan A.(2014). Delcour, Laboratory of Food Chemistry and Biochemistry, Leuven Food Science and Nutrition Research Centre (LFoRCe), KU Leuven, Leuven, BelgiumStarch/Stärke, 66, 1–16.

Keller I., Fluri P. and Imdorf A. (2005). Pollen Nutrition and Colony Development in Honey Bees. Journal of Bee World.86(1) ,3-10.

Khojasteh Shalmany S. and Shivazad M. (2006). The effect of diet prpolis supplementation on ross broilers performance. International Journal of Poultry Science, 5:84.

LeBlanc BW., Eggleston G., Sammataro D.,Cornett C., Dufault R., Deeby T. and Cyr E.(2009). Formation of hydroxymethylfurfural in domestic high-fructose corn syrup and its toxicityto the honey bee (Apis mellifera).

Menezes C., Vollet-Neto A., Leon Contrera FA., Venturieri GC. and ImperatriZ-Fonseca VL.(2013) .The role of useful microorganisms stingless bees and stingless bee Keeping. PP.153-171.

Piccini C., Antunez K .and Zunino P. (2004). An approach to the characterization of the honey bee hive bacterial flora. Apicultural Reserch: 43(3), 101-104.

Roulston,T .(2004).The effect of pollen concentration on body size in the sweat bee lasioglossim zephyrum (Hymenoperta : Apiforms). Evohutionary Ecology .16:49-65.

Rosa C.A., Lachance M.A., Silva J.O., Teixeira A.C., Marini M. M .,Antonini Y and Martinis R.P.(2003).Yest communities associated with stingless bees.FEMS Yeast Reasearch.4:271-275.

Ruiz-Matute A., Weiss M., Sammataro D., Finley J and Luz Sanz M. (2010). Carbohydrate composition of High-Fructose Corn Syrups (HFCS) used for bee feeding: effect on honey composition. Journal of Agriculture and Food Chemistry 58: 7317-7322.

Sarbojoy S. (2018). Isolation, Detection and Characterization of Aerobic Bacteria from Honey Samples of Bangladesh. Journal of biorxiv.

SAS Institue. (2000). InstitueSAS SQL Procedure User Guide, Version 9. SAS, Cary, ISBN: 13:978-158025599X, 576.

Schrezenmeir J. and Devrese M. (2001). Probiotics, prebiotics, and compartmentalization of definition. Am. Journal of Clininical Nutrition. 73, 361-364.

Stangaciu S. (1977). A Guide to propoliseorigin, uses in the hive charactevistics, Composition, properties, indications, counter -Indication. Human Uses, Aduministration and Products. Available from://www. Apiterapiapia.org/dr-stefan- stangaciu. Htm [Accessed 6 september.

Tajabadi N., Mardan M., Abdul Manap M.Y., Shuhaimi M., Meimandipour A. and Nateghi L. (2011). Detection and identification of Lactobacillus bacteria found in the honey stomach of the giant honeybee Apis dorsata. Apidologie. 42:642–64910.

Tajabadi N., Mardan M., Abdul Manap M.Y., Shuhaimi M., Meimandipour A. and Nateghi L. (2014). Identification of Lactobacillus plantarum, Lactobacillus pentosus and Lactobacillus fermentum from honey stomach of honeybee. 15;44(3):717-22.

Toth A.L.and Robinson G.E.(2005).Worker Nutrition and Division of Labor in Honey bees journal of Animal Behavior.69(2) ,427-435.

Vogel,B.2000.uberdie beziehung switchmen sussgesch mark and honey biene._z.vergl.phisip 14.273.347.

Yoshihama M.and Kimura K.(2009)Bacteria in the gur of jpanese honey bee Apis cerana Japonica, and their antagonistic effect against paenibacillus larvae, the causal agent of American foulbrood. journal of Invertebrate pathology.102:91-96.