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Original Research Article

Evaluation of Nutritional Effects of Different Levels of Enzymatic Fructose and Sugar Syrup on the Half Life and Bacterial Flora of the Bee Digestive System

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ABSTRACT

The research was carried out in a completely randomized design with 6 treatments and 6 replications in the bee incubator (Apis Mellifera Meda) from the second half of May 2019 till the end of July 2018 in Isfahan Agricultural and Natural Resources Research Center. In order to evaluate the nutritional effects of different levels of enzymatic fructose (0, 10, 20, 30, 40 and 50%) and sugar (50, 40, 30, 20, 10 and 0%) on the longevity and bacterial flora of the bee digestive system, mean comparison between experimental treatments showed that the lowest longevity and half-life between experimental levels was related to treatment with 50% fructose (P <0.05). The longevity of control treatment and treatments with 10% fructose were significant at 38.8 and 32.2 days, respectively, and the lowest feed intake was also observed at 50% fructose (P <0.05). Maximum and minimum Lactobacillus bacteria population in the bee digestive system inside cage were 10% fructose with 8.30(log CFU/g) and 50% fructose, respectively. The bacterial population of coliforms in the digestive system of bees inside the cage was the lowest with 10% fructose and the highest with 50% fructose (P <0.05).

Keywords: Incubator, Longevity, Lacto bacillus, Coliform, Digestive system, Treatments

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.

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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 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).

MATERIALS AND METHODS

Treatment and Experimental design

The experiment was carried out at the Microbiology Laboratory of Animal Science Research. Study experiments were carried out in the summer of 2019 on the European honey bee incubator (Apis Mellifera Meda) in two stages, on the longevity and amount of feed intake and microbial population of the bee at Isfahan Agricultural and Natural Resources Research Center, incubator center. Treatments included the effect of replacing different levels of fructose enzymes (0, 10%, 20%, 30%, 40%, 50%) on biological traits of bees, such as: food intake, half-life (50% mortality), longevity (100% Loss), Population Changes of Coliforms and Lactobacilli in the Bee's digestive System.

The experimental treatments investigated in this study are as follows:

Group A: fed with 1: 1 sugar syrup (50% water + 50% sugar + 0% fructose) (control group)
Group B: fed with 1: 1 sugar syrup (50% water + 40% sugar + 10% fructose)
Group C: fed with 1: 1 sugar syrup (50% water + 30% sugar + 20% fructose)
Group D: fed with 1: 1 sugar syrup (50% water + 20% sugar + 30% fructose)
Group E: fed with 1: 1 sugar syrup (50% water + 10% sugar + 40% fructose)
Group F: fed with 1: 1 sugar syrup (50% water + 0% sugar + 50% fructose)

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).

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

Mortality rate in the first incubation period

Analysis of variance 1 showed that using different levels of enzymatic fructose in the bee feeding had a significant effect on their mortality rate during the first incubation period ($P < 0.001$). Mean comparison table (2) shows that in the period 1 to 14 days, the highest mortality was observed in F treatment (containing 50% fructose replacement) and equal to 74.87% ($P < 0.05$). On the other hand, treatment A (control group) with 25% mortality had less mortality than treatments D and F (45. 54 and 74.87, respectively) ($P < 0.05$). During the first 28 days (1 to 28 days), significant differences were observed between the experimental groups in terms of number of deaths, with the lowest mortality in treatments A (control group) with 81% and treatment B (containing 10% fructose replacement) with 89% observation. It should be noted that the mortality rate in other treatments was 100%.

Table 1. Analysis of variance of the effect of different levels of enzymatic fructose on the average daily mortality of bees of different ages in the first incubation period in percentage.

Changes	**Degrees of freedom**	**average of squares**	
		1 to 14 days (%)	1 to 28 days (%)
Treatments	5	***1560.38	***312.39
Trial error	24	189.16	39.86
Total	29		

n.s: meaningless. *: Significant at the 5% probability level. **: Significant at the 1% probability level. ***: Significant at 0.01% probability level.

Table 2. Comparison of the average effect of using different levels of enzymatic fructose on the average daily mortality of bees of different ages in the first incubation period in percentage.

Experimental treatments	**1 to 14 days (%)**	**1 to 28 days (%)**
A	[c] 25.02	[c] 81.38
B	[bc]28.49	[b] 89.80
C	[bc] 43.72	[a] 100
D	[b] 45.54	[a] 100
E	[bc] 40.90	[a]100
F	[a] 74.87	[a]100
SE	2.55	1.72

a-c: In each column, meanings without similar letters were significantly different (P <0.05).
Treatments included: A: fed 1: 1 sugar syrup (50% water + 50% sugar + 0% fructose), B: fed 1: 1 sugar syrup (50% water + 40% sugar + 10% fructose) ,: C fed 1: 1 sugar syrup (50% water + 30% sugar + 20% fructose),: D fed 1: 1 sugar syrup (50% water + 20% sugar + 30% fructose), E : Fed 1: 1 sugar syrup (50% water + 10% sugar + 40% fructose), F: fed 1: 1 sugar syrup (50% water + 0% sugar + 50% fructose).

Mortality rate in the second incubation period

According to analysis of variance table 3, it was found that although the bee mortality in the second incubation period and between the ages of 1 to 7 days was not affected by the experimental treatments but fructose supplementation could have significantly affected the numbers of mortality between 1 and 28 days of age (P <0.001).

Table 3. Analysis of variance The effect of using different levels of enzymatic fructose on the average daily mortality of bees of different ages in the first incubation period in percent.

Changes	**Degrees of freedom**	**average of squares**	
		1 to 7 days (%)	1 to 14 days (%)
Treatments	5	[ns]32.40	[***]752.7
Trial error	24	14.87	55.6
Total	29		

n.s: meaningless. *: Significant at the 5% probability level. **: Significant at the 1% probability level. ***: Significant at 0.01% probability level.

Mean comparison table 4shows that at age 1 to 7 days, they received the highest mortality in group F (containing 50% fructose) with 13.6%, which were statistically different from B treatments (Containing 10% fructose) that these differences were statistically significant with treatments B (containing 10% fructose) and D (containing 30% fructose), which had a mortality rate of 7.8 and 6.4%, respectively (P <0.05). The highest mortality observed in period 1 to 14 was on average with 46% in F treatment (containing 50% fructose) which showed significant differences with other treatments (P <0.05).

Table 4. Comparison of the average effect of using different levels of enzymatic fructose on the average daily mortality of bees of different ages in the first incubation period in percent.

Experimental treatments	**1 to 7 days (%)**	**1 to 14 days (%)**
A	[ab] 10.6	[b]16.8
B	[b]7.8	[b] 13.2
C	[ab] 8.4	[b] 13.8
D	[b] 6.4	[b]22.4
E	[a]13.60	[b]19.8
F	a 74.87	[a]46.0
SE	0.715	1.385

a-c: In each column, meanings without similar letters were significantly different (P <0.05). Treatments included: A: fed 1: 1 sugar syrup (50% water + 50% sugar + 0% fructose), B: fed 1: 1 sugar syrup (50% water + 40% sugar + 10% fructose) ,: C fed 1: 1 sugar syrup (50% water + 30% sugar + 20% fructose),: D fed 1: 1 sugar syrup (50% water + 20% sugar + 30% fructose), E : Fed 1: 1 sugar syrup (50% water + 10% sugar + 40% fructose), F: fed 1: 1 sugar syrup (50% water + 0% sugar + 50% fructose).

Comparison of mean tables provided information on how experimental treatments affected mortality rates in the first and second incubation periods. These studies showed that using different amounts of enzymatic fructose in bee feeding significantly affected mortality rates so the lowest mortality was observed in the control (A treatment) which did not contain any fructose and with the increase in fructose in the experimental groups the mortality was increased so that the highest mortality was observed in the bees treated with F (1: 1 sugar syrup) (50% water + 0% sugar + 50% fructose). In this study, no significant difference in bee mortality was observed due to the use of fructose with sugar (Samataro & Weiss, 2000). However, in line with the findings of the present study, some researchers reported an increase in fructose bee mortality (Weiss, 2009; Van Engelstrop et al., 2010). The researchers said that the reason for the increase in losses due to the use of enzymatic fructose may be due to the formation of the hydroxymethyl furofural compound. This compound, which is toxic to bees, can be readily produced by heat, chemical properties of fructose, low acidity, and maintenance of fructose at high ambient temperatures and can increase mortality in bees (Van Engelstrop et al., 2010).

50% half-life and longevity of bees

Table 5 showed that half-life (time to reach 50% mortality) and longevity (time to reach 100% mortality) in bees were significantly affected by experimental treatments ($P < 0.001$). According to data provided in the mean comparison table 4-6, the lowest half-life (50% mortality) with 11.4 days in F treatment was observed (containing 50% fructose replacement) which the difference between treatment with A (control group) was 19 days and treatment B (containing 10% fructose) for 17 days was statistically significant ($P < 0.05$). Among the experimental treatments, the highest longevity was observed in the experimental treatments A (control group) with 38.8 days and treatment B (containing 10% fructose) with 32.2 days ($P < 0.05$). The table also shows a significant difference between the treatments receiving 20% fructose and higher than the control and treatment groups (containing 10% fructose) ($P < 0.05$) that these results show that by replacing fructose with white sugar in bee feed, bee life is significantly decreased ($P < 0.05$). The effects of different fructose levels on the half-life of bees were presented in Tables 5 and 6. As mentioned, the half-life of bees was 50% and the longevity of bees was affected by dietary treatments and with increasing fructose intake in the diet, the half-life of bees was significantly reduced compared to the control treatment. Contrary to our findings, early studies on the use of fructose in bee feeding indicated that bees could use this feed without any problems (Baker & Lahnner, 1974; Rinder & Baxter, 1980). But some scholars have also talked about the toxicity of this compound to bees (Jachimowicz & El Sherbini, 1975; Johnson & Johnson, 1977). (Abadi et al. 2018), using different sugars sources in bee nutrition, it was found that the survival power of fructose and glucose receiving was higher than control treatment. But the researchers found that extending the longevity of sugar-fed would be more significant than fructose-receiving (Baker & Lahnner, 1978). Another study has shown that the use of enzymatic fructose significantly reduces the half-life of worker bees, which in turn reduces the amount of honey produced (Weiss, 2009). In another study, bees that received sugar were significantly longer than those fed fructose (Sammataro & Weiss, 2000). The reason for the reduced half-life observed in bees due to the use of enzymatic fructose may be due to the oligosaccharides present in this compound (Weiss, 2009). It is found that enzymatic fructose contains high amounts of oligosaccharides such as fructosyl fructose and fructosyl glucose. These compounds are not only toxic to bees but can also cause increased mortality (Duffault et al., 2009; Ruiz Matthews et al., 2010).

Table 5. Analysis of variance of the effect of different levels of enzymatic fructose on the mean half-life of 50% and half-life of 100% (day) and average feed intake (in g) of bees.

Changes	**Degrees of freedom**	**average of squares**		
		Half-life 50%	longevity 100% (day)	feed intake (day)
Treatments	5	***33.53		***368.32
Trial error	24	7.18		24.47
Total	29			

n.s: meaningless. *: Significant at the 5% probability level. **: Significant at the 1% probability level. ***: Significant at 0.01% probability level.

Table 6. Comparison of the average effect of different levels of enzymatic fructose on the mean half-life of 50% and 100% lifetime (days) and average feed intake (in g) of bees.

Experimental treatments	Half-life 50%	longevity 100% (day)	feed intake (day)
A	[a] 19.00	[a]38.80	[a]186.01
B	[ab] 17.00	[b]32.20	[a]154.14
C	[bc]14.80	[c] 20.60	[b] 95.71
D	[bc]14.40	[c]19.20	[b]95.82
E	[bc]14.40	[c]19.90	[b] 95.18
F	[c]11.40	[c]18.20	[b]75.70
SE	0.498	0.917	4.83

a-c: In each column, meanings without similar letters were significantly different (P <0.05).
Treatments included: A: fed 1: 1 sugar syrup (50% water + 50% sugar + 0% fructose), B: fed 1: 1 sugar syrup (50% water + 40% sugar + 10% fructose) ,: C fed 1: 1 sugar syrup (50% water + 30% sugar + 20% fructose),: D fed 1: 1 sugar syrup (50% water + 20% sugar + 30% fructose), E : Fed 1: 1 sugar syrup (50% water + 10% sugar + 40% fructose), F: fed 1: 1 sugar syrup (50% water + 0% sugar + 50% fructose).

Feed intake

Information on bee feed intake during the experiment is provided in Tables 5 and 6. According to the analysis of variance (5), bee feed intake was affected by different levels of enzymatic fructose (P <0.001). table 6 of Mean comparison shows that the highest feed intake was in the control (treatment A containing 0% fructose) and treatment B (containing 10% fructose substituted white sugar) and 186 and 154 g, respectively (p < 0.05). The lowest recorded feed intake was also observed in treatments containing more than 20% fructose substituted for white sugar in their diet (P <0.05). However, there was no significant difference between the different levels of 20-50% fructose replacement for white sugar in the feed. As shown in Table 4, bee feed intake was also affected by dietary treatments, and with increasing fructose levels, feed intake was significantly reduced compared to the control group, which is consistent with research by(Karimi et al. 2019). It should be noted here that bees daily receive relatively significant amounts of carbohydrates in honey, nectar, and other carbohydrate sources such as sugar and fructose, and these carbohydrate sources of energy required for flight (Kunida et al., 2006), cellular respiration and provide physical activities such as moving and adjusting body temperature (Chapman, 1982). The observed decrease in feed intake by replacing sugar with enzymatic fructose could be due to the presence of oligosaccharide and toxic constituents in enzymatic fructose, as it was said that oligosaccharides such as fructosyl fructose and fructosyl glucose may be present in the fructose enzyme that limit feed intake by bees (Dufault et al.,1972).

Microbial populations of the digestive system

table 7 of variance analysis and table 8 of mean comparison provide information on the effect of different levels of fructose on the microbial populations of the bee digestive system. According to Table 7, the differences observed in the coliform and Lactobacillus microbial populations among the experimental treatments were not statistically significant. But the ratio of Lactobacillus to coliform population was affected by the experimental treatments such that treatment B (containing 10% fructose substituted white sugar) with treatment F (containing 50% fructose substituted white sugar) had a ratio of 16/0and 16/0, respectively so they had significant differences with each other (P <0.05).

Table 7. Analysis of variance of the effect of using different levels of enzymatic fructose on the mean microbial populations of bees in the digestive tract of different ages (log CFU/g).

Changes	**Degrees of freedom**	**average of squares**		
		Coliform	Lacto Bacillus	Ratio of Lactobacillus Populations to Coliforms
Treatments	5	[ns]0.24	[ns]0.21	[ns]0.76
Trial error	12	[ns]0.39	[ns]0.39	[ns]0.42
Total	17			

n.s: meaningless. *: Significant at the 5% probability level. **: Significant at the 1% probability level. ***: Significant at 0.01% probability level.

Table 8. Comparison of the average effect of using different levels of enzymatic fructose on the mean microbial populations of the bee digestive tract of different ages (log CFU/g).

Experimental treatments	Coliform	Lacto Bacillus	Ratio of Lactobacillus Populations to Coliforms
A	7.33	8.30	[ab]0.96
B	7.08	8.68	[ab]1.40
C	7.16	8.56	[ab]1.07
D	7.56	8.63	[ab]0.82
E	7.39	8.20	[b]0.16
F	7.85	8.02	[b] 0.16
SE	0.150	0.14	

a-c: In each column, meanings without similar letters were significantly different (P <0.05).
Treatments included: A: fed 1: 1 sugar syrup (50% water + 50% sugar + 0% fructose), B: fed 1: 1 sugar syrup (50% water + 40% sugar + 10% fructose) ,: C fed 1: 1 sugar syrup (50% water + 30% sugar + 20% fructose),: D fed 1: 1 sugar syrup (50% water + 20% sugar + 30% fructose), E : Fed 1: 1 sugar syrup (50% water + 10% sugar + 40% fructose), F: fed 1: 1 sugar syrup (50% water + 0% sugar + 50% fructose).

Tables 7 and 8 provide information on the possible effects of fructose on the microbial structure of the bee digestive system. Accordingly, none of the microbial groups of coliforms as a harmful microbial population and Lactobacillus as a useful microbial population in the gastrointestinal tract were affected by dietary treatments. However, the use of enzymatic fructose reduced the number of Lactobacillus and increased the number of coliforms. It should be noted here that environmental and nutritional factors in general are among the most important factors affecting the bee microflora (van den Bogaard, 1997). The change in microbial populations due to the use of fructose may be due to its lower acidity. Researchers have suggested that the use of compounds that reduce digestive acidity will affect the microbial population of the gastrointestinal tract (Rekiel et al., 2007). However, some researchers have stated that bee infants are able to maintain the ionic concentration of their food and thus not be affected by the acidity of their food (Bignell & Heath, 1985; Grabow et al., 1981). In this regard, it seems that any mechanism that increases Lactobacillus populations and reduces coliforms is useful for enhancing the health and performance of bees. In this regard, it seems that any mechanism that increases Lactobacillus populations and reduces coliforms is useful for enhancing the health and performance of bees. This group has the lowest number of Lactobacillus species and the lowest Formi population. Coliforms can also reduce appetite by producing phospholipase A2 compounds (Khojasteh & Shivazad, 2006), so the claim may be part of the reduction in feed intake observed in F treatment compared to control treatment.

CONCLUSIONS

The results of this experiment showed that with increasing fructose, mortality was increased and the longevity of bees decreased. Also, with increasing fructose in experimental diets, the number of coliform bacteria increased and the number of Lacto bacillus decreased.

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