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Effect of various amounts of sunflower oil in feed additives on breast tissues' functional condition, reproductivity, and productivity of honey bees

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We found that as a result of adding sunflower oil in the amount of 10 and 20 grams to the feed supplement consisting of low-fat soy flour and sugar syrup, it undergoes the dose-dependent increase of the content of saturated, monounsaturated, and mainly polyunsaturated fatty acids, both in fatty acids of total lipids and in non-esterified fatty acids. Providing bees with a feed supplement enriched with sunflower oil in the amount of 10 and 20 g leads to a dose-dependent increase in phospholipids' concentration in the breast tissue of honey bees. Simultaneously, in the phospholipids of the above tissues of I and II experimental groups, the relative content of saturated and polyunsaturated fatty acids increases, but that of monounsaturated ones decreases. In this case, the ratio of the relative content of polyunsaturated fatty acids of the ω-3 family to the polyunsaturated fatty acids of the ω-6 family decreases in the phospholipids of honey's breast tissues bees in the experimental groups I and II. The increase in the concentration of phospholipids and the relative content of polyunsaturated fatty acids of the ω-3 and especially ω-6 families leads to a dose-dependent increase in the sorption capacity of breast tissues of honey bees of the experimental groups I and II. Meanwhile, the content of iron, zinc, copper, chromium, lead, and cadmium increases in the breast tissues of honey bees of the experimental group II. Changes in the content of phospholipids, their fatty acid composition, and sorption capacity of breast tissues of honey bees of the experimental groups I and II are accompanied by changes in the reproductive capacity of bee queens and honey productivity worker bees. In particular, the queens of these groups increase egg production, and worker bees improve honey productivity.
Keywords: honey bees, feed supplement, fatty acids, phospholipids, egg-laying of bee queens, honey productivity.

Introduction

The analysis of the scientific sources on the topic shows that the amount and composition of fatty acids in feed impacts the fatty acid composition and functional activity of phospholipids of cell membranes through phospholipids directly and very quickly (Couture & Hulbert, 1995; Arien et al., 2015; Ma et al., 2015; Arien et al., 2018). Significantly, the fatty acid content of phospholipids of cell membranes is the main factor influencing the intensity of the transition of various compounds, including heavy metals and various forms of fatty acids, by active and passive transportation, into the tissues of bees. In turn, the content of phospholipids and their fatty acid composition in bees' tissues depends on the functioning of their nervous, immune, reproductive systems, and the oxidation process. The latter reacts markedly to the amount and composition of fatty acids in the feed (Gätschenberger et al., 2013; Arien et al., 2015). The problem of fatty acids in food - bee tissues - tissues' functional activity is as follows. Mentioned fatty acids in honey bees' feed and tissues are involved in reproductive capacity and productive traits (Arien et al., 2015; Ma et al., 2015; Rabiee et al., 2015; Wu et al., 2017). Depending on the amount and composition, fatty acids can change the supply of bees with energy, structural, and biologically active material (Hulbert & Abbott, 2011; Hulbert et al., 2014; Rabiee et al., 2015). This is caused by the fact that bee tissues can synthesize only saturated and monounsaturated long-chain fatty acids with enzyme systems' help. Bee tissues cannot synthesize long-chain polyunsaturated fatty acids (Hulbert, 2010; Arien et al., 2015; Arien et al., 2018). Therefore, such polyunsaturated fatty acids as linoleic and linolenic should enter their body with food. The primary sources of essential linoleic and linolenic acids in the food for bees are feed supplements (Hulbert & Abbott, 2011; Hulbert et al., 2014; Arien et al., 2015). The polyunsaturated fatty acids mentioned above are dominant in the feed's fatty acid composition (Arien et al., 2015; AL-Kahtani, 2017). A common sign of deficiency of α-linoic and α-linolenic acids in the body of bees is a decrease in growth rate, efficiency of absorption of feed nutrients, suppression of immunity, and

reduced productive traits and reproductive capacity (Hulbert & Abbott, 2011; Arien et al., 2015; Ma et al., 2015; Vishchur et al., 2016; Arien et al., 2018; Kovalskyi et al., 2018; Kovalchuk et al., 2019; Vishchur et al., 2019; Piven et al., 2020).
There is no data in the literature on phospholipids' content and their saturated, monounsaturated, and polyunsaturated fatty acids in honey bees' tissues depending on their amount and composition in the feed. There is also no information about the functional state of bee tissues, taking into account phospholipids' content and their fatty acid composition. These facts determine the relevance of the topic of the research.
The purpose of the research is to discover the linkage of phospholipids content and their fatty acid composition and sorption capacity of the bee breast tissues reproductive capacity and productivity of honey bees depending on the amount and composition of fatty acids in the feed supplement.

Materials and methods

Experimental studies were conducted on clinically healthy honey bees of Carpathian breed (*Apis mellifera carpatica*) in the spring and summer season in a private apiary in Zastavna district Chernivtsi region.
According to the analog principle, three groups of bee families were formed (3 bee families in each). Bee families of a control group were fed with a feed supplement consisting of 100 g of deoiled flour from natural soya beans of Chernivetska-9 variety and 100 g of sugar syrup (sugar to water ratio – 1:1) once a week for 36 days. In addition to this feed supplement, bees of families I and II of experimental groups received sunflower oil in 10 and 20 g per bee colony every week, respectively. During the experiment, the reproductive ability of females and honey productivity of worker bees were controlled.
Studies of egg-laying by queen bees were performed by the method (Lavrehin & Pankova, 1983). To implement this, the number of sealed broods was recorded every 12 days using a special frame-grid with a square size of 5×5 cm. The amount of obtained commercial honey was determined by weighing the honeycombs selected from the nests before and after pumping.
After feeding, samples of honey bees for laboratory testing were selected. The breast honey bees' tissues underwent testing of phospholipids' content and their fatty acid composition, the concentration of heavy metals, and sorption capacity. The content of phospholipids and their fatty acid composition in the test material was determined using the gas-liquid chromatography according to Y.F. Rivis (Rivis, 2017), and the content of heavy metals - by atomic absorption spectrophotometry according to I. Havezov and D. Tsaliev (Havezov & Calev, 1983). The studied tissue's sorption capacity was determined with the method of staining created by M.V. Jakovlev (Jakovlev, 1958).
In particular, phospholipids' content in the studied biological material was determined by extraction of lipids with a mixture of chloroform-methanol (2:1 by volume). Chloroform-free lipids were subjected to the thin layer chromatography on silica gel. The obtained lipid fractions, including phospholipids, were determined by photoelectrocolometer.
The fatty acid composition of the obtained phospholipids was determined by dissolving them in a non-polar solvent and adding to a solution of sodium methylate in methyl alcohol. The methyl esters of fatty acids obtained in such a way were introduced into the gas-liquid chromatographic apparatus's evaporator. Separation of fatty acid methyl esters was performed on a "Chrom-5" chromatograph ("Laboratorni pristroje", Praha).
The sorption capacity of the tissue under investigation was determined by washing it in Ringer's saline solution, staining the tissue with a solution of neutral red, its extraction, and photocolorimetry.
The content of heavy metals (iron, zinc, copper, chromium, nickel, lead, and cadmium) in the biological material studied was determined on a C-115 PK atomic absorption spectrophotometer. To do this, samples of breast tissue were ashed in a muffle furnace at a temperature of 450–500 °C. That ash was dissolved in 10 ml of 10 % HCl. The ash's obtained acidic solutions were processed with spectrophotometer at a strictly defined wavelength on an atomic absorption spectrophotometer.
Statistical processing of the obtained results was performed using the computer program Microsoft Excel. The Student's ratio figured out the probability of intergroup differences in results.

Results and discussion

We found that the natural feed supplement, which consists of non-fat soy flour and sugar syrup, contains a certain amount of fatty acids of total lipids and non-esterified fatty acids, easily accessible to the honey bee body (Table 1). The addition of sunflower oil, which contains in its composition 61.8 % of dietary linoleic acid, in the amount of 10 and 20 g to the feed supplement mentioned above, results in a significant increase of the content of lauric, myristic, pentadecanoic, palmic, palmitoleic, stearic, oleic, linoleic, linolelaidic, arachinic and eicosaic acids both in composition of the fatty acid of total lipids and non-esterified fatty acids.
The increase in the content of fatty acids of total lipids and non-esterified fatty acids in the feed supplement leads to a substantial increase in the concentration of phospholipids in the breast tissue of honey bees of experimental groups I and II compared with breast tissue of honey bees of the control group (Table 2). At the same time, phospholipids in the honey bees' breast tissues in the first and second experimental groups undergo an increase in the relative concentration of saturated and polyunsaturated fatty acids and a decrease in monounsaturated ones compared with phospholipids in breast tissues of honey bees in the control group (Table 2).

Table 1. The fatty acid content in feed supplements without and with sunflower oil, g/kg of natural weight

Fatty acids and their code	Feed supplement (CD)	CD + 10 g of sunflower oil	CD + 20 g of sunflower oil
Fatty acids of total lipids			
Lauric, 12: 0	0.01	0.06	0.11
Myristic, 14: 0	0.22	0.11	0.20
Pentadecanoic, 15: 0	0.04	0.22	0.41
Palmic, 16: 0	0.51	2.56	4.63
Palmitoleic, 16: 1	0.04	0.22	0.40
Stearic, 18: 0	0.38	1.95	3.53
Oleic, 18: 1	2.65	14.22	26.08
Linoleic, 18: 2	6.82	34.34	62.20
Linolelaidic, 18: 3	0.23	1.17	2.12
Arachinic, 20: 0	0.04	0.21	0.37
Eicosaic, 20: 1	0.03	0.17	0.30
including non-esterified fatty acids			
Lauric, 12: 0	traces	0.002	0.004
Myristic, 14: 0	0.001	0.006	0.009
Pentadecanoic, 15: 0	0.002	0.010	0.016
Palmic, 16: 0	0.02 4	0.114	0.224
Palmitoleic, 16: 1	0.002	0.010	0.017
Stearic, 18: 0	0.014	0.087	0.159
Oleic, 18: 1	0.148	0.694	1.227
Linoleic, 18: 2	0.320	1,412	2,814
Linolelaidic, 18: 3	0.010	0.048	0.098
Arachinic, 20: 0	0.002	0.009	0.014
Eicosaic, 20: 1	0.001	0.007	0.011

Rise of the relative content of saturated fatty acids is observed due to fatty acids with an even number of carbon atoms in the chain (in experimental groups I and II, respectively, up to 23.91 and 23.84 versus 22.60%) an odd number of carbon atoms in the chain (0.30 and 0.31 versus 0.28), as well as polyunsaturated fatty acids of ω-3 family (21.29 and 21.35 against 21.13) and ω- 6 family (18.48 and 18.93 versus 17.17%). The decrease in monounsaturated fatty acids' relative content is mainly due to fatty acids of the family ω- 9 (in experimental groups I and II, respectively, up to 34.91 and 34.41 versus 37.85 %). Notably, phospholipids in honey bee breast tissues in the first, second experimental groups, compared with phospholipids in breast tissue in the control group, demonstrated a reduction in the ratio of relative content of family ω-3 polyunsaturated fatty acids and family of ω-6 polyunsaturated fatty acids (Table. 2). The data mentioned above indicate a significant decrease in the structural organization and functional activity of the cell membranes of honey bees' breast tissue.

Table 2 shows that phospholipids in breast tissues of the honey bee in experimental groups I and II, compared with phospholipids in breast tissue in the control group, probably undergo the increase in the relative content of such polyunsaturated fatty acids as eicosadic and eicosatric-arachidonic but decrease in such monounsaturated fatty acids as oleic. The phospholipids in honey bee breast tissues in the second experimental group demonstrate a rise in the relative concentration of such saturated fatty acids as caprylic, capric, lauric, myristic, pentadecanoic, and palmitic and such polyunsaturated fatty acids as linoleic, eicosatric, docosadic, and docosatetraic. The data above point to a significant increase in phospholipids' content, which are included in the structure of cell membranes of honey bee breast tissues. Simultaneously, due to the deterioration of the fatty acid composition of phospholipids, the functional activity of the cell membranes of the breast tissues of honey bees is somewhat reduced.

An increase in phospholipids concentration and the relative content of polyunsaturated fatty acids of the ω-3 family and especially ω-6 family leads to an increase in the sorption capacity of breast tissues of honey bees of the first (6.2 ± 0.17 units of extinction) and second (6.7 ± 0.20 , $p<0.05$) experimental groups, compared with the breast tissues of the control group (5.6 ± 0,20 units of extinction). This indicates an increase in the permeability of the breast tissues of honey bees for activated and inactivated compounds.

The growth of sorption capacity of the breast tissues of honey bees in the experimental group II, compared with the breast tissues of honey bees in the control group, causes the increase in the concentrations of iron, zinc, copper, chromium, plumbum, and cadmium (Table 3). These mineral elements may be more absorbed into the tissues of the digestive tract. All facts mentioned above demonstrate a significant water and water-soluble substances permeability increase of breast tissues of honey bees in experimental group II compared with the honey bees' breast tissues in control one.

Table 2. The level of phospholipids (g/kg of raw weight) and their fatty acids composition (%) in the breast tissue of honey bees (M ± m, n = 3)

Phospholipids, fatty acids and the code of the latter	A control group (feed supplement - CD)	And experimental (CD + 10 g of sunflower oil)	Research II (CD + 20g of sunflower oil)
Phospholipid	5.61 ± 0.159	6.00 ± 0.067	6.13 ± 0.047*
Caprylic, 8:0	0.15 ± 0.006	0.17 ± 0.006	0.18 ± 0.003*
Capric, 10:0	0.23 ± 0.006	0.25 ± 0.003	0.26 ± 0.006*
Lauric, 12:0	0.32 ± 0.006	0.34 ± 0.003	0.35 ± 0.003*
Myristic, 14:0	0.52 ± 0.014	0.57 ± 0.009	0.57 ± 0.006*
Pentadecanoic, 15:0	0.28 ± 0.006	0.30 ± 0.003	0.31 ± 0.003*
Palmic, 16:0	9.71 ± 0.237	10.82 ± 0.383	10.93 ± 0.302*
Palmitoleic, 16:1	0.97 ± 0.026	1.11 ± 0.052	1.16 ± 0.046*
Stearic, 18:0	11.43 ± 0.442	11.51 ± 0.430	11.29 ± 0.433
Oleic, 18:1	37.64 ± 0.525	34.70 ± 0.428 *	34.20 ± 0.373**
Linoleic, 18:2	9.45 ± 0.202	10.04 ± 0.124	10.24 ± 0.149*
Linolelaidic, 18:3	5.24 ± 0.136	5.20 ± 0.081	5.11 ± 0.066
Arachinic, 20:0	0.24 ± 0.009	0.25 ± 0.003	0.26 ± 0.003
Eicosaic, 20:1	0.21 ± 0.006	0.21 ± 0.006	0.21 ± 0.003
Eicosadic, 20:2	0.27 ± 0.006	0.30 ± 0.003 *	0.31 ± 0.003 **
Eicosatric, 20:3	1.55 ± 0.070	1.75 ± 0.038	1.81 ± 0.043 *
Arachidonic, 20:4	4.86 ± 0.089	5.25 ± 0.074 *	5.38 ± 0.061 **
Eicosapentanic, 20:5	1.35 ± 0.037	1.36 ± 0.022	1.37 ± 0.037
Docosadic, 22:2	1.04 ± 0.020	1.14 ± 0.029	1.19 ± 0.032 *
Docosatric, 22:3	1.26 ± 0.035	1.27 ± 0.047	1.28 ± 0.042
Docosatetraic, 22:4	2.83 ± 0.046	3.04 ± 0.066	3.12 ± 0.062 *
Docosapentanic, 22:5	4.83 ± 0.101	4.84 ± 0.124	4.83 ± 0.107
Docosahexaic, 22:6	5.62 ± 0.150	5.59 ± 0.113	5.64 ± 0.123
Total fatty acid content	100	100	100
including saturated	22.88	24.21	24.15
monounsaturated	38.82	36.02	35.57
polyunsaturated	38.30	39.77	40.28
ω-3/ω-6	1.23	1.15	1.13

Note: in this and the following tables * - p<0.05; ** - p<0.01; *** - p<0.001.

Table 3. The content of heavy metals in the breast tissues of honey bees, g· 10^{-3}/kg of raw mass (M ± m, n = 3)

Heavy metals and their symbols	A control group (feed supplement - FS)	Experimental group I (FS + 10 g sunflower oil)	Experimental group II (FS + 20 g sunflower oil)
Iron, Fe	48.48 ± 1.065	51.47 ± 0.655	52.61 ± 0.455*
Zinc, Zn	30.43 ± 0.979	33.30 ± 0.681	34.15 ± 0.528*
Copper, Cu	3.06 ± 0.092	3.30 ± 0.046	3.59 ± 0.093*
Chrome, Cr	4.07 ± 0.139	4.50 ± 0.110	4.60 ± 0.069*
Nickel, Ni	5.26 ± 0.156	5.64 ± 0.067	5.79 ± 0.110
Plumbum, Pb	1.19 ± 0.061	1.37 ± 0.043	1.44 ± 0.035*
Cadmium, Cd	0.08 ± 0.006	0.10 ± 0.006	0.12 ± 0.009*

As a result, the change in the content of phospholipids, their fatty acid composition, and sorption capability of honey bee breast tissues in experimental groups I and II, compared with the breast tissues of honey bees in the control group, cause changes in the reproductive capacity of bee queens and worker bees honey productivity. Moreover, compared with queen bees of the control group, queens of experimental groups I and II demonstrate oviposition growth within the experiment period (Table 4).

Table 4. Reproductive capacity of queens, eggs per day (M ± m, n= 3)

A control group (feed supplement - FS)	Experimental group I (FS + 10 g sunflower oil)	Experimental group II (FS + 20 g sunflower oil)
Preparatory period, April 5		
201.2±10.89	206.9 ± 16.35	202.3 ± 17.49
	Experimental period, April 17	
757.4±19.12	830.4 ± 24.99	896.7 ± 16.11**
	Experimental period, April 29	
856.0±18.56	956.1 ± 24.59 **	1108.0 ± 20.17***
	Experimental period, May 11	
893.1±14.50	1018.0 ± 4.76 **	1163.8 ± 24.84***
	Total for the experimental period, April 17 - May 11	
2506.5	2804.5	3168.5

Relatedly, an increase in the honey productivity of worker bees of the first (14.5 ± 0.40 kg, $p<0.01$) and II (15.7 ± 0.34, $p<0.001$) experimental groups, compared with the control group worker bees (12,4±0.36 kg), is observed.

Conclusions

Due to mixing the feed supplement consisting of low-fat soy flour and sugar syrup with sunflower oil in the amount of 10 and 20 grams, the content of saturated, monounsaturated, and mainly polyunsaturated fatty acids both in fatty acids of total lipids and in non-esterified fatty acids increases depending on the amount of oil.

Providing bees with a feed supplement enriched with sunflower oil in the amount of 10 and 20 g leads to a dose-dependent increase in phospholipids' concentration in the breast tissue of honey bees. Simultaneously, in the phospholipids of the mentioned above tissues of bees of experimental groups I and II, the relative content of saturated and polyunsaturated fatty acids increases but that of monounsaturated ones decreases. Herewith, the ratio of the relative content of polyunsaturated fatty acids of the ω-3 family to the polyunsaturated fatty acids of the ω-6 family decreases in the phospholipids of honey's breast tissues bees in the experimental groups I and II.

The increase in the concentration of phospholipids and the relative content of polyunsaturated fatty acids of the ω-3 and especially ω-6 families leads to a dose-dependent increase in the sorption capacity of breast tissues of honey bees of the experimental groups I and II. Meanwhile, the content of iron, zinc, copper, chromium, lead, and cadmium increases in the breast tissues of honey bees of the experimental group II.

Changes in the content of phospholipids, their fatty acid composition, and sorption capacity of breast tissues of honey bees of the experimental groups I and II are accompanied by changes in the reproductive capacity of bee queens and honey productivity worker bees. In particular, the queens of these groups increase egg production, and worker bees improve honey productivity.

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