NEW NUTRITIVE SUPPLEMENTS FOR FEEDING MELLIFERA BEES IN THE DEFICIT PERIODS OF COLLECTION IN NATURE

[1]Toderaş Ion, [1]Cebotari Valentina, [1]Ungureanu Laurenţia, [1]Buzu Ion, [1]Gheorghiţă Cristina, [2]Floquet Sebastien, [3]Gulea Aurelian, [4]Rudic Valeriu, [2,3]Fuior Arcadie

1. Institute of Zoology of the Academy of Sciences of Moldova.
2. University of Versailles St-Quentin-En-Yvelines, France.
3. State University of Moldova.
4. Institute of Microbiology and Biotechnology of the ASM

Rezumat

Scopul prezentei lucrări a fost testarea în hrana albinelor a biomasei microalgelor acvatice *Scenedesmus quadricauda Turp, Scenedesmus apiculatus Hortob, Oocystis borgei Snow* şi elaborarea, în baza acesteia, a unor procedee de hrănire a familiilor de albine în perioadele deficitare de cules melifer în natură, sfârşitul iernii şi începutul primăverii (februartie – martie). Cercetările au fost efectuate pe familiile de albine *Apis mellifera Carpatica* la stupina experimentală a Institutului de Zoologie al Academiei de Ştiinţe a Moldovei. La sfârşitul lunii februarie, au fost formate 5 loturi similare de familii de albine, câte 13-15 familii în fiecare lot. Fiecărei familii de albine i-au fost administrate o singură dată, câte 200 g de amestec de pudră de zahăr cu miere în proporţie de 7:3, de consistenţa unei paste gelatinoase dense în formă de turtă, câte o turtă la fiecare ramă cu albine. În lotul I (martor), albinele au primit în hrană doar pastă nutritivă din pudră de zahăr+mere; în lotul II, albinele au primit în hrană pastă nutritivă îmbogăţită cu suplimentul nutritiv cunoscut „*Apispir”*, în cantitate de 2 ml de soluţie cu concentraţia de 10% (200 mg de substanţă activă) la 1 kg de pastă; în loturile III, IV şi V, albinele au primit ca hrană pastă nutritivă, îmbogăţită cu biomasa microalgelor acvatice, respective: *Scenedesmus quadricauda, Scenedesmus apiculatus* şi *Oocystis borgei Snow,* în cantitate de 10 ml suspensie cu concentraţia de 2,0% (200 mg substanţă uscată) la 1 kg de pastă. La intervalul de 100 zile de la data hrănirii albinelor cu pastă nutritivă (care a coincis cu primul cules), la fiecare familie din loturile experimentale, individual, au fost evaluate: prolificitatea mătcii, cantitatea de puiet căpăcit, puterea familiei, rezistenţa la boli, viabilitatea puietului, cantitatea de ceară crescută pe faguri, cantitatea de păstură şi de miere acumulate în cuib. Rezultatele cercetării au demonstrat că, hrănirea familiilor de albine în perioadele deficitare de cules melifer în natură cu amestec nutritiv din zahăr-pudră+miere în raport de 7:3, îmbogăţit cu suplimente biologic active din

biomasa microalgelor acvatice *Scenedesmus quadricauda, Scenedesmus apiculatus* şi *Oocystis borgei Snow*, contribuie la creşterea, comparativ cu lotul martor, a: prolificităţii mătcilor - cu 7,8-10,3% (td=3,5-5,2; P<0,01-0,001); cantităţii de puiet căpăcit – cu 7,7-9,3% (td=3,4-4,7; P<0,01-0,001); puterii familiei de albine – cu 7,1- 9,3% (td=3,0-3,5; P<0,01); rezistenţei familiilor la boli – cu 1,8-3,8% (td=1,6-3,6; P<0,1- 0,01); viabilităţii puietului din cuib – cu 1,2-1,7% (td=2,2- 2,9; P<0,05-0,01); cantităţii de ceară crescute pe faguri – cu 13,3-36,7% (td=2,9-6,4; P<0,01-0,001); cantităţii de păstură acumulate în cuib – cu 15,5- 27,6% (td=3,4- 8,4; P<0,01-0,001); cantităţii de miere acumulate în cuib la primul cules – cu 28,0- 38,9% (td=7,2- 9,9; P<0,001). În baza cercetărilor efectuate au fost elaborate procedee noi de hrănire a familiilor de albine în perioadele deficitare de cules melifer în natură şi obţinute brevete de invenţie (MD 1061 Y 2016.08.31; MD 1062 Y 2016.08.31; MD 1079 Y 2016.10.31).
Cuvinte cheie: albine, hrănire, procedee, suplimente bioactive, microalge acvatice.
Depus la redacţie 03 noiembrie 2017

Adresa pentru corespondenţă: Ion Toderaş, Institutul de Zoologie al Academiei de Ştiinţe a Moldovei, str. Academiei, 1, MD-2028 Chişinău, Republica Moldova;
Email: *iontoderas@yahoo.com*; tel. +373 22 739 809.

Introduction

In modern beekeeping, the problem of organic bee nutrition during periods of poor harvesting in nature is a permanent concern for beekeepers, specialists and researchers. The most vulnerable period to ensure optimal nutrition and bee family maintenance is considered the end of winter - early spring (February - March). During this period, the reserves of natural food from the bee family's nest are depleted and in the bees' body there is a deficiency of bioactive nutrients, especially carbohydrates, proteins, microelements, vitamins, which have a decisive role in the physiological processes of activity vital part of the bee organism, determining the reproductive capacity and subsequent development of the bee family as a whole [3, 4, 5, 24].

In order to compensate for the inadequacy of nutrients in bee nourishment during periods of poor harvesting in nature, most beekeepers feed the bee families with sugar syrup, which, with the exception of carbohydrates, contains a large number of biologically active substances. Consequently, the feeding of bees with sugar syrup is not complete but unbalanced, with a negative impact on the functions of the hypopharyngeal glands of the working bees, as well as the quality of the royal jelly, which directly influences the oogenesis activity and the queen's queue, as well as the pace of development and the quality of the brood. To mitigate this impact, identifying accessible sources of biologically active substances to enrich nutritional supplements in bee ration during periods of poor harvesting in nature becomes an extremely important and current issue.

In recent decades biologists have drawn attention to the biomass of mono- and pluricellular microalgae as important sources of biologically active substances. Among these, the most studied were the microalga Chlorella vulgaris [14, 34, 35] and the pluricellular microalgae (cyanobacteria) *Spirulina platensis* [12, 15, 19]. Research has shown that Chlorella vulgaris microalgae biomass contains an important set of biologically active substances. According to some authors [14], Chlorella is called the "energy and vitality supplement" with therapeutic properties, improving the health of the body in general and fortifying the immune system, in particular, increases the body's resistance against infections. This microalgae rich in beta-carotene is able to remove pesticide residues from the body, ingested from food, extract mercury deposits, being a powerful detoxifying agent. Testing the suspension of the Chlorella vulgaris microalgae biomass in bees' food has helped to increase the growth rate of colonies by 17.0-22.4% [11].

Among the pluricellular algae species, *Spirulina platensis* [1, 2, 17, 18, 19, 20, 21] was studied the most. For over 20 years this filamentous pluricellular cyanophilic microalgae was explored as a food source. The World Health Organization and the 3rd International Congress of Food Sciences and Technologies have unconventionally defined Spirulina as an essential source of up to 50 bioactive substances that ensures the normal development of vital processes in the human and animal body.

For feeding bee families during periods of poor harvesting in nature, researchers have developed a range of nutrient mixtures based on the use of various organic and inorganic substances, such as: sugar syrup + pollen + skimmed cow's milk + casein + heteroauxin + $CoCl_2$ + $MnSO_4$ [41], honey + sugar-powder + skimmed cow's milk + sunflower groats + synthetic amino acids [40], sugar syrup + carnitine chloride suspension [36], powdered sugar + soybean flour + powdered skimmed milk + dry beer yeast [13], designed to help optimize nutrition. To strengthen the vigor and resistance to bee families, some specialists have proposed enriching nutritional supplements with biomass and Bifidocobacterium globosum strains + Streptococcus faecium + carbohydrate + aluminum oxide and hydroxide + ascorbic acid [37], sugar syrup + suspension of the Bacillus subtilis strain [38] and strains of Micomicetes [22]. The shortcomings of these processes are expressed by the fact that the ingredients in the mixtures are expensive and their efficiency is low because a lot of the proposed ingredients are difficult to digest for bees and also they are easily oxidizable which causes disturbances in the function of the digestive tract of bees.

In beekeeping, other processes for stimulating the growth of bee families by feeding with sources of biologically active substances are known, in particular with a mixture of 50% sugar syrup enriched with *Spirulina platensis* (Nordst.) Geitl CALU-835 [2. 3]. The disadvantage of this process is the low efficiency because the cells of this cyanophyte microalgae are coated with a relatively thick protective film which stagnates the process of digestion by bees of nutrients from biomass and also the sugar syrup can not be used during the winter when the atmospheric temperatures are low.

Among the known methods of enriching nutritional supplements with biologically active substances, the closest solutions, according to the technical essence and the result obtained are the feeding methods of bee families *Apis mellifera*: MD 475 Z 2012.09.30 [25], MD 476 Z 2012.09. 30 [26] and MD 477 Z 2012.09.30 [27], patented in 2012 by the Institute of Zoology of the Academy of Sciences of Moldova. These include feeding bees in the spring with a mixture of 1% mass of the biomass extract of the *Spirulina platensis* microalgae CNM-CB-02 and the 50% sugar syrup taken in a ratio of 1: 500, respectively, the biomass of this cyanophyte pluricellium microalgae being obtained by cultivation in the presence of coordinating compounds such as monochloracetate Zn (II) tetrahydrate - $Zn(CH_2ClCOO)_2{\bullet}4H_2O$, chromium and potassium salt - $KCr(SO_4)_2{\cdot}12H_2O$ and Fe (III) selenite hexahydrate $FeSeO_3{\bullet}6H_2O$

administered in the 30 -35 mg / L in one of the first three days of cultivation. The above-mentioned coordinate compounds increase the penetration of living cells, which improves the assimilation of bioactive substances in the nutritional supplement. Feeding bees with nutrition supplements enriched with biologically active substances is carried out in an amount of 100 ... 130 ml on a beehive frame every 2 days for two weeks. The disadvantage of these processes lies in the fact that the technology of obtaining the *Spirulina platensis* microalgae biomass extract grown in the presence of coordinating compounds is too complicated and costly and the mixture of sugar syrup enriched with the bioavailable supplement can not be used during the winter or early spring due to its high humidity, which releases a large amount of vapor in the nest while the bees are in the wintering yard.

For these reasons, researchers from the Institute of Zoology of the Academy of Sciences of Moldova together with the State University of Moldova [7, 28], in collaboration with the University of Versailles St-Quentin-En-Yvelines, France [6, 29] have proposed the direct use of coordination compounds as a supplement to enrich the nutrient mixture with biologically active substances, in particular as important sources of deficient microelements in nature, thus developing new bee-feeding processes in the shorter periods of harvesting in nature (MD 850 Z 2015.08.31 [28] and MD 4438 B1 2016.10.31 [29]). Research has shown that enriching nutritional supplements with coordinating compounds contributes to the stimulation of the reproductive functions of the queen and the vital activity of the bee families as a whole, in particular, it contributes to the increase of the queen's prolificity, the amount of brood, the colony's power, the viability of the brood, the increased amount of wax, the amount of pasture and honey accumulated in the nest [6, 7]. At the same time, the disadvantage of these processes lies in the fact that the coordination compounds provide feed for bees with only some rare microelements. In these coordination compounds are missing a whole range of biologically active substances such as proteins, carbohydrates, lipids, amino acids, enzymes, vitamins, antioxidants, etc.

In this context, the researchers of the Institute of Zoology of the Academy of Sciences of Moldova [8, 9, 30-33] proposed the use in the bee feeding of the biomass of aquatic microalgae as a source of biologically active substances, exclusively organic, less expensive and quite accessible to bees.

Therefore, the purpose of this paper was to test bee feeding of aquatic microalgae biomass, such as: *Scenedesmus quadricauda*, *Scenedesmus Apiculatus*, *Oocystis borgei* and the development of bee-feeding procedures during the deficient harvesting periods in nature (February - March).

Materials and methods

The research was carried out on *Apis mellifera Carpatica* bee families at the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova. In order to achieve the proposed goal, an experimental plan was developed which included feeding the bee families at the end of the winter, during the poor collection time in nature, when atmospheric temperatures were low, with a nutritional paste prepared from a mixture of sugar powder and honey in proportion 7: 3 and bioactive supplements. As bioactive supplements, the biomasses of *Scenedesmus quadricauda*, *Scenedesmus Apiculatus* and *Oocystis borgei* microalgae were mixed with the paste in a 10 ml suspension of 2% to 1 kg of the paste mixture, and feeding bee families with the enriched nutritional paste was made by distributing it in the form of disks placed in the nest above the honeycomb frames. On each honeycomb frame was put a disk, each weighing 200 g.

The biologically active supplements that we call "*Scenecuadri*", "*Apiculatus*" and "*Borgesnow*" are a 2% suspension of biologically active algae, yellowish-green algae, the dry substance of which contains 23-25% up to 47-49% of proteins, presented by the entire set of essential and non-essential, immunogenic and proteinogenic acids, from 30-35% to 40-46% of carbohydrates, from 8.1-9.2% to 11.9-12.2% of lipids, vitamins, micro-macro-elements and other important bioactive substances. In 100 mg of dry substance, the supplements contained 0.27-0.31 mg of beta-carotene as one of the main antioxidant and catalyst components of regenerative processes of the cells of reproductive tissue of the queen and the lactosynthesis of working bees. Since the above-mentioned monocellular aquatic microalgae are coated with a relatively thin protective film, biologically active substances from biomass were accessible for digestion in the bee's digestive tract.

To assess the efficiency of bee feeding procedures with the above-mentioned nutritional supplements, at the end of February, a series of comparative test experiments were carried out on bee families grouped in 5 similar groups, 13-15 families in each group, according to the scheme in Table 1.

Table 1. Scheme of test experiments in bee feeding of nutrient supplements enriched with biologically active substances from the biomass of aquatic algae.

Group	The number of bee families in the group (N)	The composition of the nutritional supplement and the name of the algae grown for biomass	Amount and concentration of the biomass solution at 1 kg of paste	Amount of active substance per 1 kg of paste
I (reference)	14	Energy paste = powdered sugar + honey (7: 3)	-	-
II	15	Energy paste + *Spirulina platensis* (*Apispir*)	2 ml of 10%	200 mg
III	13	Energy paste + *Scenedesmus quadricauda (Scenecuadri)*	10 ml of 2%	200 mg
IV	13	Energy paste + *Scenedesmus Apiculatus (Apiculatus)*	10 ml of 2%	200 mg
V	13	Energy paste + *Oocystis borgei (Borgesnow)*	10 ml of 2%	200 mg

Each bee family was given once, 200 grams of paste (one disk) per beehive. In lot I (control), the bees were fed with nutritive paste prepared only from 7: 1 honey powder mixture; In group II - the closest solution, the bees received nutrition paste prepared from a mixture of honey powder with enriched nutritional supplement "*Apispir*" [24] in 2 ml of the 10% solution (200 mg of active substance) per 1 kg of paste; In lot III, the bees received as nutritive paste food prepared from the mixture of sugar powder with honey, enriched with the biomass, the *Scenedesmus quadricauda* aquatic microalgae, hereinafter referred to as the "*Scenecuadri*" bioactive additive, in a quantity of 10 ml of a suspension of 2.0 % (200 mg of dry substance) per 1 kg of paste;

In lot IV, the bees received as nutritive paste food prepared from a mixture of honey powder, enriched with biomass, the *Scenedesmus Apiculatus* aquatic microalgae, hereinafter referred to as the "*Apiculatus*" bioactive additive, in a quantity of 10 ml of a suspension of 2.0 % (200 mg of dry substance) per 1 kg of paste; and in lot V - the bees received as nutritive paste food prepared from the mixture of honey powder, enriched with biomass *Oocystis borgei* aquatic microalgae, hereinafter called "*Borgesnow*" bioactive additive, in the amount of 10 ml of a suspension of the concentration of 2, 0% (200 mg dry matter) per 1 kg of paste.

At 100 days from the date of feeding the bees with the bioactive nutritional supplement (which coincided with the first harvest), the main morpho-productive breeding characters (queen prolificity, brood quantity) and development (family power), disease resistance and viability of the brood, as well as the productivity of the amount of waxes increased on the combs, the amount of pasture and honey accumulated in the nest of the families according to the methods developed by us [10] the zootechnical norm regarding the bee-keeping of the bee families, the breeding and certification of beekeepers' material, approved by the Government Decision of the Republic of Moldova no. 306 of 28.04.2011 [16]. The data obtained in the experiments were statistically processed using the computerized software "STATISTICA-6" and their certainty was assessed, according to the variometric biometric statics, according to Plockinski, N.A., 1989 [39].

Results and discussions

Research data shows that use in bees feeding of the aquatic biomass of monocellular algae, such as *Scenedesmus quadricauda*, *Scenedesmus Apiculatus* and *Oocystis borgei*, has a stimulating effect on bee's vital activity and, as a result, on their productivity (Table 1).

It has been found that biologically active substances contained in nutritional supplements enriched with aquatic microalgae biomass indirectly caused a stimulatory impact on the reproductive functions of the queen (oogenesis), contributing to the growth of the layed eggs number and the amount of brood and, as a result, to the development of bee family. Because the queen does not consume the nutritional supplement administered to the nest, but is permanently nourished with royal jelly by the working bees, we can say that the biologically active substances in the nutritional supplements cause a stimulatory impact on the lactogenic functions of the hypopharyngeal glands of the nurse-bees and on the qualitative composition of the royal jelly, thus stimulating the reproductive functions of the queen.

As a result, the prolificity of the queens from bee families of the III-V groups, which received nutritional supplements enriched with biomass of aquatic microalgal, increased significantly compared to those in group I (control) with 125-164 eggs / 24 hours or 7.8-10.3% (td = 3.5 and 5.2; $P < 0.01$ and $P < 0.001$). The highest prolificity was recorded in the bee families of the IV and V batches, which received nutritional supplements enriched with the biomass of algae *Scenedesmus Apiculatus* and *Oocystis borgei*, respectively 1757 and 1725 eggs / 24 hours respectively significantly higher compared to 164 and 132 eggs / 24 hours or 10.3 and 8.3% (td = 5.2 and 4.6; $P < 0.001$) of the control group. More obviously, the differences between the prolificity of the bee families in the experimental lots compared to the control group can be seen in Figure 1.

Table 2. Results of bee-feeding test of nutritional supplements enriched with biologically active substances of biomass of aquatic algae.

Group	The number of bee families in the group (N)	Average value of character first harvest (M ± m)	Difference to Group I			Difference to Group II		
			d_1	%	t_d	d_1	%	t_d
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Prolificacy of the queen, eggs / 24 hours								
I (reference)	14	1593 ± 25	-	-	-	-25	1,5	0,7
II	15	1618 ± 26	+25	1,6	0,7	-	-	-
III	13	1718 ± 25	+125	7,8	$3,5^{**}$	100	6,2	$2,8^{**}$
IV	13	1757 ± 19	+164	10,3	$5,2^{***}$	139	8,6	$4,3^{***}$
V	13	1725 ± 14	+132	8,3	$4,6^{***}$	107	6,6	$3,6^{**}$
The amount of brood, hundred cells								
I (reference)	14	191,5 ± 3,1	-	-	-	-2,7	1,4	0,6
II	15	194,2 ± 3,2	+2,7	1,4	0,6	-	-	-
III	13	206,2 ± 3,0	+14,7	7,7	$3,4^{**}$	+12,0	6,2	$2,7^{*}$
IV	13	209,3 ± 2,2	+17,8	9,3	$4,7^{***}$	+15,1	7,8	$3,9^{***}$
V	13	206,9 ± 1,6	+15,4	8,0	$4,4^{***}$	+12,7	6,5	$3,5^{**}$
Colony power, kg								
I (reference)	14	3,22 ± 0,05	-	-	-	-0,03	0,9	0,5
II	15	3,25 ± 0,04	+0,03	0,9	0,5	-	-	-
III	13	3,52 ± 0,07	+0,30	9,3	$3,5^{**}$	+0,27	8,3	$3,4^{**}$
IV	13	3,45 ± 0,06	+0,23	7,1	$3,0^{**}$	+0,20	6,1	$2,9^{**}$
V	13	3,48 ± 0,07	+0,26	8,1	$3,3^{**}$	+0,23	7,1	$2,9^{**}$
Disease resistance,%								
I (reference)	14	88,9 ± 0,8	-	-	-	-1,5	1,7	1,6
II	15	90,4 ± 0,5	+1,5	1,7	1,6	-	-	-
III	13	90,5 ± 0,6	+1,6	1,8	1,6	+0,1	0,1	0,1
IV	13	92,3 ± 0,5	+3,4	3,8	$3,6^{**}$	+1,9	2,1	$2,7^{*}$
V	13	92,0 ± 0,5	+3,1	3,5	$3,3^{**}$	+1,6	1,8	$2,3^{*}$
Viability of the brood ,%								
I (reference)	14	90,0 ± 0,3	-	-	-	-1,1	1,2	$3,1^{**}$
II	15	91,1 ± 0,2	+1,1	1,2	$3,1^{**}$	-	-	-
III	13	91,1 ± 0,4	+1,1	1,2	$2,2^{*}$	0,0	0,0	0,0
IV	13	91,2 ± 0,3	+1,2	1,3	$2,9^{*}$	+0,1	0,1	0,3
V	13	91,5 ± 1,3	+1,5	1,7	1,1	+0,4	0,4	0,3
The amount of wax, kg								
I (reference)	14	0,30 ± 0,01	-	-	-	-0,01	3,2	0,7
II	15	0,31 ± 0,01	+0,01	3,3	0,7	-	-	-
III	13	0,34 ± 0,01	+0,04	13,3	$2,9^{**}$	+0,03	9,7	$2,1^{*}$

Table 2 (Continued).

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
IV	13	0,41 ± 0,02	+0,11	36,7	5,0***	+0,10	32,2	5,0***
V	13	0,39 ± 0,01	+0,09	30,0	6,4***	+0,08	25,8	5,7***
The amount of pasture, hundreds of cells								
I (reference)	14	90,1 ± 2,1	-	-	-	-7,6	7,8	2,5*
II	15	97,7 ± 2,1	+7,6	8,4	2,6*	-	-	-
III	13	104,1 ± 3,6	+14,0	15,5	3,4**	+6,4	6,6	1,5
IV	13	115,0 ± 2,1	+24,9	27,6	8,4***	+17,3	17,7	5,8***
V	13	110,5 ± 1,5	+20,4	22,6	7,9***	+12,8	13,1	5,0***
The amount of honey, kg								
I (reference)	14	10,74 ± 0,35	-	-	-	-1,03	8,7	2,0*
II	15	11,77 ± 0,37	+1,03	9,6	2,0*	-	-	-
III	13	13,75 ± 0,24	+3,01	28,0	7,2***	+1,98	16,8	4,6***
IV	13	14,92 ± 0,24	+4,18	38,9	9,9***	+3,15	26,8	7,3***
V	13	14,90 ± 0,25	+4,16	38,7	9,7***	+3,13	26,6	7,0***

It is important to note that the prolificity of the bee families in the experimental groups receiving nutrition supplements enriched with biomass of algae significantly increased not only compared to the control group but also to those in bee families in group II prototype) that received the nutritional supplement enriched with biologically active substances of the *Spirulina platensis* microalgae. Thus, the beehive families of the experimental groups III, IV and V, which received nutrition supplements enriched with biologically active supplements "*Scenecuadri*", "*Apiculatus*" and "*Borgesnow*", had higher proliferation compared to those in group II, which received the feed enriched with the *Apispir* supplement with 100, 139 and 107 eggs / 24 hours or 6.2 respectively; 8.6 and 6.6% (td = 2.8; 4.3 and 3.6; P <0.01; P <0.001 and P <0.01).

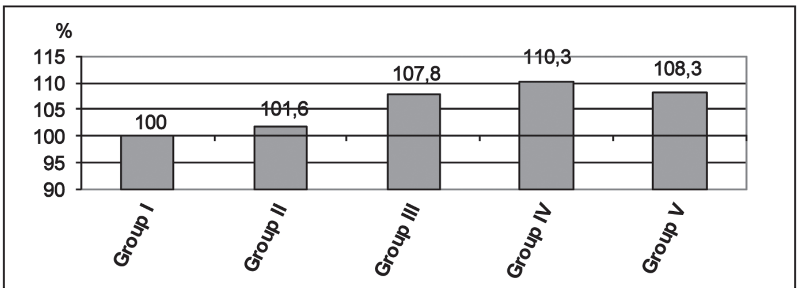

Fig. 1 The prolificity of queens from bee families in the experimental groups, compared to the control group.

As a consequence of the increase of the prolificity of the queens, in lots with the bee families, which benefited from the nutritive supplements enriched with biologically active substances from the aquatic microalgae biomass, there was a significant increase, compared to the control group, of the quantity of broodstock (Figure 2). Thus, according to the data obtained, the amount of brood of the bee families of groups III, IV and V, which received in food the mixture enriched with biologically active nutritional supplements "*Scenecuadri*", "*Apiculatus*" and "*Borgesnow*" constituted 206,2 - 209.3 hundred cells, which is 14.7 - 17.8 hundred cells or 7.7 - 9.3% higher, compared to the control group (td = 3.4 - 4.7, P <0.01 - 0.001). The largest quantity of brood (209.3 ± 2.2 hundred cells) was recorded in experimental group IV bee families, which received the nutritional mixture enriched with the biomass of *Scenedesmus Apiculatus*, with 17.8 hundred cells or 9.3% higher than in the control group (td = 4.7, P <0.001).

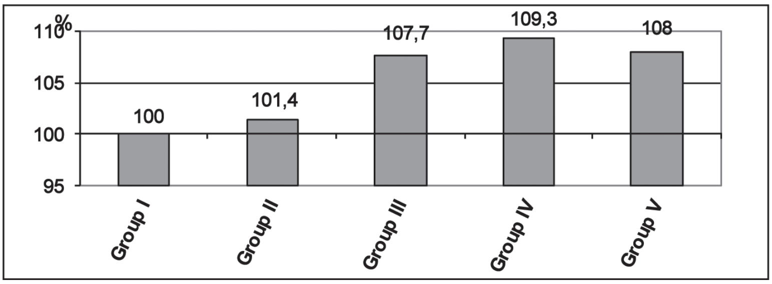

Fig. 2 The amount of brood of bee families in the experimental groups, compared to the control group.

It is worth mentioning that the biologically active substances from the aquatic microalgae biomass have caused bee families an increase in the quantity of brood and compared with the bee families of the second prototype group II (the closest solution) who received the supplement nutritional "*Apispir*". Thus, the bee families in experimental groups III, IV and V exceeded, by this character, the families of bees in lot II, respectively, by 12.0; 15.1 and 12.7 hundred cells or 6.2; 7.8 and 6.5% (td = 2.7; 3.9 and 3.5; P <0.05; P <0.001 and P <0.01).

Increasing the prolificity of the queens and the amount of brood in the bee families, which received nutritional supplements enriched with biomass of water algae, indirectly led to the essential increase of the total amount of bee populated in the nest, called in the literature the power of the family (Figure 3).

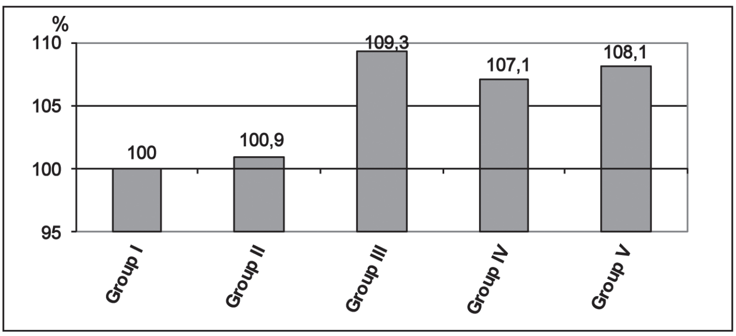

Fig. 3. The power of the bee families in the experimental groups, compared to the control group.

It was conceded that in the bee families of the experimental groups, which received nutritional supplements enriched with the biomass of the aquatic microalgae, the average strength of the colony consisted of 3.45 - 3.52 kg, which is higher compared to the families of bees in the control group with 0.23-0.30 kg or 7.1-9.3% (td = 3.0-3.5, P <0.001).

The highest quantity of working bees was recorded in the bee families of group III, which received a nutritional mixture enriched with the biomass of the microalga *Scenedesmus quadricauda*, constituting 3.52 ± 0.07 kg, the highest, compared to the group control with 0.30 kg or 9.3% (td = 3.5; P <0.001).

It is worth mentioning that the biologically active substances in the aquatic microalgal biomass caused an increase in the number of bees populated in the nest, not only compared to the control group, but also compared to the bee families of the prototype II lot (the nearest solution) who received the nutritional supplement "*Apispir*". Thus, the bee families in experimental lots III, IV and V exceeded, by this character, the bee families in lot II, respectively, by 0.27; 0.20 and 0.23 kg or 8.3; 6.1 and 7.1% (td = 3.4, 2.9 and 2.9, P <0.01).

Bee families at different times of the year are attacked by many pathogens of microbial, virotic, micotic, parasitic origin, etc. Naturally, the bee's immune system opposes resistance to pathogens. Bee colony has a hygienic instinct, expressed by the attachment of pathogens, their isolation and elimination, as well as of foreign bodies, impurities, larvae and dead bees. The rate of elimination of dead larvae on the beehives correlates directly with the immunity of bees, so in the normative documents of the specialty, the hygienic instinct of the bee family is called resistance to disease.

The hygienic instinct (disease resistance) of the bee family is determined by the immune system and influenced by environmental factors, such as: food harvesting and quality (both natural and extra), bee-care measures, and prophylaxis of diseases, etc. To strengthen the resistance of bee families to bee families, beekeepers apply different bee feeding methods and means of nutrition supplements that contain, along with the energy-protein components, biologically active substances - stimulating the vital functions of bees that help increase their immunity [13, 40, 41].

The results of the research have shown that bee feeding with the biologically active nutritional supplements *Scenecuadri*, *Apiculatus* and *Borgesnow* at the end of winter - during the poor collection period in nature, contributed to the increase of the values of the disease resistance characters (Fig. 4).

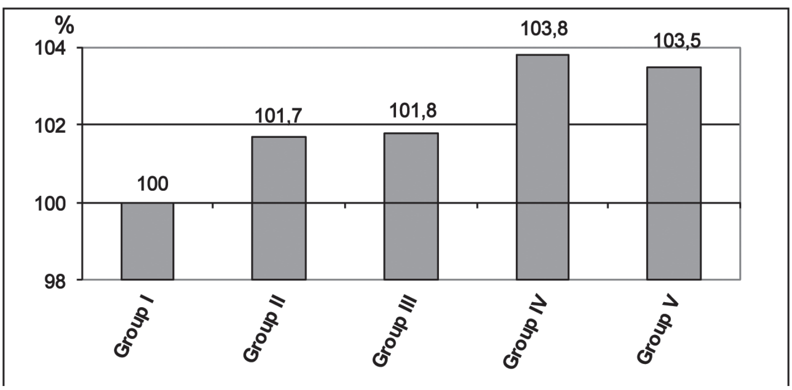

Fig. 4. Resistance to bee families from the experimental groups, compared to the control group.

It has been found that biologically active substances in nutritional supplements have had a stimulating impact on immune modulating functions, accelerating the hygienic instinct of bees, contributing to the strengthening of disease resistance.

Thus, the bee families in experimental groups IV and V, which received nutritional mixtures enriched with the biomass of aquatic microalgae *Scenedesmus Apiculatus* and *Oocystis borgei*, had a significantly higher disease resistance compared to those in the control group, 3.6 -3.3% (P & lt; 0.01).

At the same time, the bee families of group III, who received nutritional mixtures enriched with biomass, the microalgae *Scenedesmus quadricauda*, had only a higher resistance to disease than those in the control group.

It should be noted that the bee families in experimental groups IV and V, which received the nutritional supplements enriched with algae *Scenedesmus Apiculatus* and *Oocystis borgei* algae, had a higher disease resistance compared to those in the prototype II lot, which had received the nutritional mixture enriched with the bioactive "*Apispir*" supplement with 2.1 and 1.8% ($P < 0.05$).

It was found that nourishing bee families during the poor winter collection with bioactive nutrient supplements enriched with aquatic microalgae biomass ensure a fairly high brood viability at 91.1-91.5 percentage points. Due to the action of biologically active substances in nutritional supplements enriched with aquatic algae biomass, the working bees in the experimental families had a more intense vital activity, indirectly contributing to the increase of viability of the brood by feeding them with royal jelly, honey and pasture.(Fig 5).

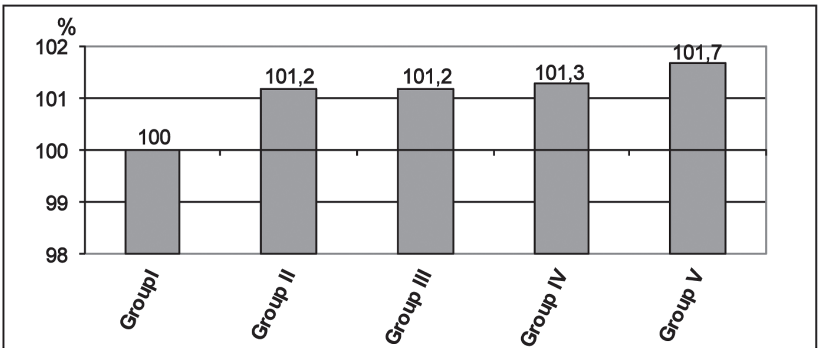

Fig. 5. Viability of the bee families of the experimental groups, compared to the control group.

Thus, the viability of brood of bee families in Sections II, III and IV significantly increased compared to Group I (control) by 1.2-1.3% (td = 2.2-3.1, $P<0.05$ and $P<0.01$). Given that the variability of these biological features (disease resistance and brood viability) is very narrow, the significance of these differences (small at first sight as absolute dimensions) is quite large and corresponds to a high level of certainty, according to the probability theory predictions without error after Stiudent [39]. Despite the fact that brood viability and disease resistance are mostly hereditary biological properties (h2 = 0.7-0.8), however, research data show that feeding bees with biologically active substances from the biomass of aquatic microalgae contributes, certainly to strengthen the immunity and vigor of bee families as a result - to increase their vital activity.

The result obtained in increasing disease resistance and viability is determined by the presence in the nutritional supplements administered of biologically active substances, in particular proteins presented by the whole set of essential and non-essential acids, immune and proteinogenic, lipid, micro-macro- vitamins including beta-carotene, one of the main antioxidants and catalysts of regeneration processes of the reproductive tissues of the queen and lactosynthesis of working bees. Some of the biologically active substances are part of hormones and enzymes from the hemolimph, catalyzing important functions of the bee's vital activity, having stimulating, immune-modulating and antioxidant properties. They help to improve the penetration of organic tissue cells, participate in the process of hemocyte regeneration and stimulate the immune system of the body. Therefore, biologically active substances in nutritional supplements have activated the physiological functions of bees, which has led to the strengthening of immunity, increasing the resistance to diseases and the viability of the brood, resulting in a decrease in the incidence of morbidity and bee mortality.

Due to the rich content of biologically active substances, the nutritional supplements administered to the bees during the poor time of harvesting in nature, stimulated the secretory activity of the bee's wax-producing gland causing an escalating increase in the amount of wax quantity in the nest (Fig. 6).

Thus, within 100 days of the feeding of bees with nutritional supplements, the bee families in experimental groups III, IV and V, which received the nutrition supplements enriched with the biomass of algae *Scenedesmus quadricauda*, *Scenedesmus Apiculatus* and *Oocystis borgei* increased 0.34 ± 0.01 - 0.41 ± 0.02 kg of wax, which is more compared to the bee families in the control group, with 0.04 - 0.11 kg or 13.3- 36.7% (td = 2.9-5.0; P <0.01 and P <0.001).

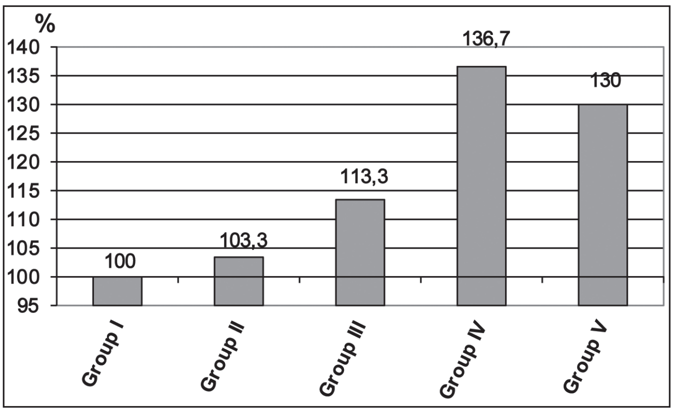

Fig. 6. Increased amount of wax in the bee family's nest of the experimental groups compared to the control group.

The highest increase in wax was recorded in the nests of bee families in the experimental Lot IV, which received a nutrient mixture enriched with the biomass of *Scenedesmus Apiculatus* microalgae. They exceeded their congeners in the control group by 0.11 kg or 36.7% (td = 5.0; P <0.001), on the amount of wax in the nest. It is important to note that bee families in experimental groups III, IV and V, which received nutrient mixtures enriched with biologically active supplements "*Scenecuadri*", "*Apiculatus*" and "*Borgesnow*" in food, exceeded the amount of wax in the nest , not only their congeners in the control group, but also the bee families of the prototype II lot, which received the *Apispir* bioactive additive, respectively, with 0.03; 0.10 and 0.08 kg or 9.7; 32.2 and 25.8% (td = 2.1, 5.0 and 5.7, P <0.05 and P <0.001).

Due to the higher power, disease resistance and increased viability of the brood, the bee colonies in experimental groups III, IV and V, which received biologically active nutritional supplements obtained from the biomass of aquatic microalgae, gained a more pronounced accumulation in the nest of apiculture products, which is the end result for which the bee families are grown and exploited. It has been found that biologically active substances from the *Scenedesmus quadricauda*, *Scenedesmus Apiculatus* and *Oocystis borgei* aquatic microalgae have indirectly provoked a stimulating impact on the functions of the vital activity, in particular on the working activity of the bees at the first harvest, contributing to the nesting of some higher quantities of apiculture products, pasture and honey.

Thus, the amount of pasture accumulated in nest by bee families in experimental groups III, IV and V, which received nutrition supplements enriched with the biologically active supplements "*Scenecuadri*", "*Apiculatus*" and "*Borgesnow*", was significantly higher , compared to the bee families in the control group, with 14.0-24.9 hundred cells or 15.5-27.6% (td = 3.4-8.4; P <0.01 and P <0.001) , (Figure 7).

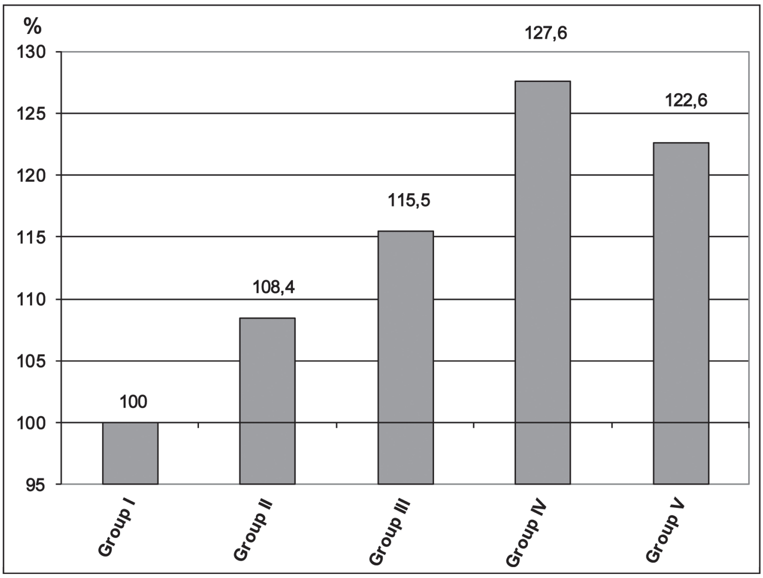

Fig. 7. The amount of pasture accumulated in the nest of bee families of the experimental groups compared to the control group.

The data show that the largest amount of pasture was accumulated in the beehive of experimental group IV, the bees receiving a nutrient-enriched mixture with the microalgae *Scenedesmus Apiculatus*, constituting an average of 115.0 ± 2.1 hundred cells , being significantly higher compared to bee families in the control group with 24.9 hundred cells or 27.6% (td = 8.4; P <0.001).

It is important to note that the bee families in experimental groups IV and V, which received nutritional mixtures enriched with the biomass of the scalds and aphidulatus and *Oocystis borgei*, exceeded the amount of pasture in the nest, not just their congeners in the control group, but also the bee families of the prototype II lot, which received a nutritional mixture enriched with the biologically active "*Apispir*" supplement with 17.3 and 12.8 hundred cells or 17.7 and 13.1% (td = 5,8 and 5.0, P <0.001).

Finally, our research has shown that the greatest influence of the biologically active substances contained in the nutritional supplements obtained from the aquatic microalgae biomass has been exerted on the function of accumulation of the amount of honey in the nest as one of the most important biological properties of Mellifera bees, which provide the food supply for its own colony and basic apiculture production for the hive (Fig.8).

Thus, 100 days after the feeding of the bees with nutritive mixtures, the amount of honey accumulated in the nest by the bee families in experimental groups III, IV and V, which received the biologically active supplements "*Scenecuadri*", "*Apiculatus*" and "*Borgesnow*" constituted 13.75 ± 0.24 - 14.92 ± 0.24 kg, being significantly higher, compared to the bee families in the control group, with 3.01 - 4.18 kg or 28.0 -38.9% (td = 7.2-9.9, P<0.001).

The results of the research have shown that the greatest capacity for the honey accumulation in the nest was manifested in the bee families of the Experimental Lot IV, who received the nutritional mixture enriched with the biomass of the aquatic microalga *Scenedesmus Apiculatus*. After this, the bee families in lot IV significantly exceeded their congeners from the control group with 4.18 kg or 38.9% (td = 9.9, P <0.001).

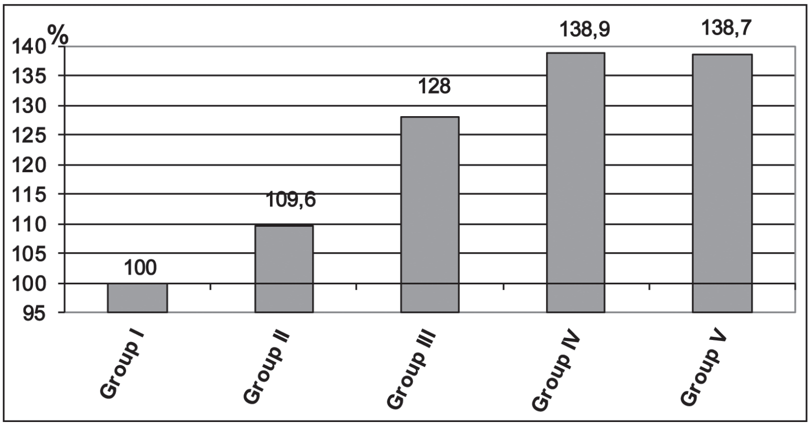

Fig. 8. The amount of honey accumulated in the nest of bee families of the experimental groups compared to the control group.

It should be noted that bee families from experimental groups III, IV and V, which received nutritional supplements enriched with the biomass of *Scenedesmus quadricauda*, *Scenedesmus Apiculatus* and *Oocystis borgei*, simultaneously exceeded significantly on the amount of honey accumulated in the nest, not just their congeners in the control group, but also the bee families of the prototype II lot, which received in the feed the biologically active nutritional supplement "*Apispir*", respectively, with 1.98; 3.15 and 3.13 kg or 16.8; 26.8 and 26.6% (td = 4.6, 7.3 and 7.0, P <0.001).

Therefore, the technical result of the use of aquatic microalgae biomass in bee feeding consists in the stimulation of oogenesis functions and the laying egg capacity of queens, the increase in the quantity of broodstock and the number of massively hatched bees, which has led to a quantitative increase in the power of bee families, higher productivity. The result is due to the increased digestibility and accessibility of biomass nutrients, given that *Scenedesmus quadricauda*, *Scenedesmus Apiculatus* and *Oocystis borgei* are coated with a thin film and the biomass has a rich content of biologically active substances, proteins, carbohydrates, lipids, essential amino acids, micro-macro-elements, antioxidants (beta-carotene), which have a catalytic role in the metabolism of nitrogen-containing substances in working bees, participate in the synthesis of enzymes, improves the vial activity of bees, having direct influence on their flight intensity, by activating nectar and pollen picking functions as well as secretory functions of the wax-producing glands, stimulates the functions of the reproductive system of the queen, by activating oogenesis and laying egg capacity.

All this determine, to a great extent, the activation of the physiological processes of the body of all members of the bee family, starting with the queen - founder of the social community (the nest) and finishing with the working bees, the vitality of which depends the power of the bee families and the collection capacity of apiculture products' precursors (nectar, pollen) and their productive potential as a whole.

On the basis of the researches carried out, new methods of feeding bee families [30-32] were developed during the late winter and early spring (February-March), deficient in nature when atmospheric temperatures are low. These processes ensure the bees' needs in easily digestible and affordable biological nutrients that are less expensive and easier to cultivate, which contribute to increasing the efficiency of the use of nutrients from biomass, stimulating the prolificity of the queens, accelerating the development of the power and increasing the productivity of bee families *Apis mellifera* overall.

Conclusions

1. Feeding bee families during periods of poor harvesting in nature (end of winter - February and beginning of spring - March) with a 7:3 sugar-powder and honey nutrition mixture, enriched with biologically active supplements from aquatic microalgae biomass *Scenedesmus quadricauda*, *Scenedesmus Apiculatus* and *Oocystis borgei*, contribute to the increase of a series of indicators compared to the control group: the prolificity of the queens - by 7.8; 10.3 and 8.3%, respectively (td = 3.5; 5.2 and 4.6; P <0.01 and P <0.001); the amount of brood - by 7.7; 9.3 and 8.0% (td = 3.4; 4.7 and 4.4; P <0.01 and P <0.001); bee family power - by 9.3; 7.1 and 8.1% (td = 3.5; 3.0 and 3.3; P <0.01); the resistance of families to diseases - by 1.8; 3.8 and 3.5% (td = 1.6; 3.6 and 3.3; P <0.1 and P <0.01); the brood viability in the nest - by 1.2; 1,3 and 1,7% (td = 2,2; 2,9 and 1,1; P <0,05; P <0,01 and P> 0,1); of the amount of wax grown on combs - by 13.3; 36.7 and 30.0% (td = 2.9; 5.0 and 6.4; P <0.01 and P <0.001); the amount of herds gained in the nest - by 15.5; 27.6 and 22.6 (td = 3.4; 8.4 and 7.9; P <0.01 and P <0.001); the amount of honey accumulated in the nest at the first harvest - by 28.0; 38.9 and 38.7% (td = 7.2; 9.9 and 9.7; P <0.001).

2. Nutrient mixtures enriched with microalgae biomass of *Scenedesmus quadricauda*, *Scenedesmus Apiculatus* and *Oocystis borgei* are more effective compared to the biologically active "*Apispir*" supplement obtained from *Spirulina platensis* microalgae biomass at increasing the prolificity by 6.2-8.6% (td = 2.8-4.3; P <0.01 - 0.001); the amount of capped brood - by 6.2-7.8% (td = 2.7-3.9; P <0.05 - 0.001); power of colony - by 6.1-8.3% (td = 2.9-3.4; P <0.01); resistance to disease - by 1.8-2.1% (td = 2.3-2.7; P <0.05); the amount of wax grown on combs - by 9.7-32.2% (td = 2.1-5.7; P <0.05 - 0.001); of the amount of pasture accumulated in the nest - by 6,6-17,7% (td = 1,5-5,8, P> 0,1 and P <0,001) and the amount of honey accumulated in the nest - by 16,8- 26.8% (td = 4.6-7.3; P <0.001).

3. On the basis of the researches carried out, new methods of feeding bee families (MD 1061 Y 2016.08.31, MD 1079 Y 2016.10.31, MD 1079 Y 2016.10.31) were developed during the deficit periods of harvesting in nature, when the atmospheric temperatures are low and bee feeding cannot be done with sugar syrup.

Confirmation

The work was carried out within the framework of the fundamental institutional project 15.817.02.12F "Diversity, structure and functioning of natural and anthropized fauna complexes in the context of strengthening the national security strategy of the Republic of Moldova" financed by the Supreme Council for Science and Technological Development of the Academy of Sciences of Moldova.

References

1. Bulimaga V., Rudic V., Zosim L., Chiriac T., Turtă C., Prodius D., Şova S., Mereacre V. Procedeu de obţinere a biomasei de *Spirulina platensis*. Brevet de invenţie nr. MD 3128 G2 2006.08.31, Chişinău, 2006a.

2. Bulimaga V., Rudic V., Zosim L., Chiriac T., Turtă C., Prodius D., Melnic S., Mereacre V. Procedeu de obţinere a biomasei cianobacteriei *Spirulina platensis*. Brevet de invenţie nr. MD 3129 G2 2006.08.31, Chişinău, 2006b.

3. Cebotari V., Toderaş I., Buzu I. The use of biologically active substances for strengthening of rezistance to diseases of honeybee colonies *Apis mellifera*. University of Agricultural Sciences and Veterinary Medicine, Iasi, Romania. International Scientific Symposium: Modern zootehny, factor of sustainable development – Centennial 1912 - 2012. Lucrări Ştiinţifice, Cotaţie CNCSIS cu B^+, vol. 57, Seria Zootehnie, ISSN 1454-7368, Iaşi, 2012, pag. 39-43.

4. Cebotari V., Toderaş I., Buzu I., Rudic V. The role of „*Apispir*+Zn" biostimulator in increasing of productivity of *Apis mellifera* bee colonies. University of Agricultural Sciences and Veterinary Medicine Iasi. Scientific Papers. Series Animal Science. Vol. 59, Impact CNCSIS B^+, ISSN 2284-6964; ISSN-L 1454-7368. Iasi, 2013a, p. 103-107..

5. Cebotari V., Toderaş I., Buzu I., Rudic V. Use of chrome trace for vital activities functions stimulations of *Apis mellifera* bee colonies. University of Agronomic Sciences and Veterinary Medicine of Bucharest. Scientific Papers. Series D. Animal Science. Vol. LVI, Impact CNCSIS B^+, code index 1020, ISSN 2285-5750, ISSN-L 2285-5750. Bucharest, 2013b, p. 73-77.

6. Cebotari V., Toderaş I., Gulea A., Buzu I. The microelement molybdenum (*Mo*) in nutritional supplement for bees. In: International Scientific Symposium „Modern animal husbandry – food safety and durable development" at the University of Agricultural Sciences and Veterinary Medicine from Iasi. Scientific papers. Animal Science. Ed. „Ion Ionescu de la Brad". Vol. 66(22), Iaşi, 2015, p. 56-63, ISSN 1454-7368.

7. Cebotari V., Buzu I., Toderaş I., Gulea A., Postolachi O., Toderici V., Gliga O. Influence of some organic coordination compounds containing *Co* and *Bi* on development morpho-productive characters of the bee families. In: International Conference „Agriculture for Life, Life for Agriculturer" at the *University of Agronomic Sciences and Veterinary Medicine of Bucharest*. Scientific papers. Series D. Animal Science. Ed. „CERES" Publ. House. Vol. LVIII, ISSN 2285-5750, Bucharest, 2015, p. 251-258.

8. Cebotari V., Buzu I., Gliga O., Postolachi O. New nutritional supplements for bees during deficient harvesting perionds (*Oocystis borgei Snow*). In: International Scientific Symposium „Modern animal husbandry – food safety and durable development" at the Univrsity of Agricultural Sciences and Veterinary Medicine Iasi. Scientific papers. Animal Science. Ed. „Ion Ionescu de la Brad". CD-Rom, Iaşi, 2016, p. 100-107, ISSN 2284-6964.

9. Cebotari V., Buzu I., Postolachi O., Gliga O. Testing of the nutrient supplement enriched with biomass of aquatic algaie in the bees feed (*Scenedesmus quadricauda*). In: International Conference „Agriculture for Life, Life for Agriculturer" at the *University of Agronomic Sciences and Veterinary Medicine of Bucharest. Scientific papers. Series D. Animal Science.* Ed. „CERES" Publ. House. Vol. LIX, Bucharest, 2016, p. 85-90. ISSN 2285-5750.

10. Cebotari V., Buzu I. Zootechnical norms regarding the honeybee colonies evaluation, breeding and certification of genetic material in beekeeping. In: Contemporary Science Association. Proceedings of the 1st International Animal Health Science Conference. The Beekeeping Conference. Addleton Academic Publishers, New York, (Bucureşti), 2010, Library of Congress Control Number, pag. 26-30.

11. Eremia N., Bahcivanji M., Zagareanu A. Study of algal *Chlorella vulgaris* suspencion on grouth and productivity of bees families. University of Agricultural Sciences and Veterinary Medicine Iasi. Scientific Papers. Animal Sciences. Vol. 59 (18), Iaşi, 2013, p. 148-152.

12. Ionov V.A., Basova M.M. Use of blue-green micro-seaweed *Spirulina platensis* for the correction of lipid haemostatic disturbances in patients with ischemic heart disease. Вопросы питания, 2003, 72:28-31.

13. Lazăr Şt. Bioecologie şi tehnologie apicolă. Ed. „Alfa", Iaşi, 2002, 536 p.

14. Luca C. Chlorella. Sănătate cu dr. Cătălin Luca. Copyringht, 2012. http: //dr.cătălin.luca.ro.

15. Mazo V.K., Gmoshinskii I.V., Zilova I.S. Microalge Spirulina in human nutrition. Вопросы питания. 2004; 73:45-53.

16. Normă zootehnică privind bonitarea familiilor de albine, creşterea şi certificarea materialului genitor apicol, aprobată prin Hotărârea Guvernului nr. 306 din 28.04.2011 (M.O. nr. 78-81 din 13.05.2011, art. 366), Chişinău, 2011.

17. Rudic V., Zosim L., Bulimaga V., Chiriac T., Ciumac D., Turtă C., Prodius D. Procedeu de obţinere a biomasei de *Spirulina platensis*. Brevet de invenţie nr. MD 3101 G2 2006.07.31, Chişinău, 2006a.

18. Rudic V., Bulimaga V., Djur S. Mediu nutritiv pentru cultivarea cianobacteriei *Spirulina platensis*. Brevet de invenţie nr. MD 3417 G2 2007.10.31, Chişinău, 2007.

19. Rudic V., Bulimaga V., Chiriac T. et al. New nutraceuticals from *Spirulina*. International Conference on Exploatation of agricultural and food industry by-products and waste material trough the application of modern processing techniques. Institute of Bioengineer. and Environmental Protection–S.C. BIOING S.A., Bucharest, 2008a, p.26-29.

20. Rudic V., Toderas I., Gudumac V.et al. New remedies for bees. Akademos, Chişinău, 2008b, 4(11), p.81-83.

21. Rudic V., Chiriac T., Bulimaga V., Zosim L., Gulea A., Poirier D., Ciapurina L., Ciumac D., Toderaş I. Procedeu de obţinere a biomasei cianobacteriei *Spirulina platensis*. Brevet de invenţie nr. MD 3171 G2 2006.10.31, Chişinău, 2006b.

22. Starciuc N., Sârbu T., Bugneac V., Burţeva S., Postolachi O., Cebotari V. Soil micromicetes antagonism against some pathogenes of *Apis mellifera*. The 12th International Symposium „Prospects for the 3rd Millenium Agriculture", Universitatea de Ştiinţe Agricole şi Medicină Veterinară. Book of abstracts. Section 9: Veterinary Medicine – Clinical sciences, Cluj-Napoca, România, 2013, p. 246

23. Toderaş I., Rudic V., Derjanschi V., Roman R., Gudumac V., Chiriac T., Bogdan V., Sicora D. Procedeu de stimulare a creşterii familiilor de albine şi sporirea productivităţii lor. Brevet de invenţie MD 2061 G2 2003.07.31, Chişinău, 2003.

24. Toderaş I, Rudic V., Gulea A., Cebotari V., Buzu I. Influenţa remediilor organice bioactive de generaţie nouă asupra activităţii vitale a familiilor de albine *Apis mellifera*. Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţele Vieţii, ISSN 1857-064X, categoria B, Nr. 3 (324), Chişinău, 2014, p. 4-15.

25. Toderaş I., Rudic V., Cebotari V., Bogdan V., Gulea A., Bulimaga V., Buzu I., Chiriac T., Arcan E. Procedeu de hrănire a familiilor de albine *Apis mellifera*. Brevet de invenţie MD 475 Z 2012.09.30. Buletinul Oficial de Proprietate Industrială, nr. 2, Chişinău, 2012a, p. 28.

26. Toderaş I., Rudic V., Cebotari V., Bogdan V., Gulea A., Bulimaga V., Buzu I., Chiriac T., Railean N. Procedeu de hrănire a familiilor de albine *Apis mellifera*. Brevet de invenţie MD 476Z 2012.09.30. Buletinul Oficial de Proprietate Industrială, nr.2, Chişinău, 2012b, p.28-29.

27. Toderaş I., Rudic V., Cebotari V., Bogdan V., Gulea A., Bulimaga V., Buzu I., Chiriac T., Silistrari A. Procedeu de hrănire a familiilor de albine *Apis mellifera*. Brevet de invenţie MD 477 Z 2012.09.30. Buletinul Oficial de Proprietate Industrială, nr. 2, Chişinău, 2012c, p. 29-30.

28. Toderaş I., Cebotari V., Gulea A., Buzu I., Toderici V., Bulimestru I., Gliga O., Railean N., Silistrari A. Procedeu de hrănire a familiilor de albine *Apis mellifera.* Brevet de invenţie nr. MD 850 Z 2015.08.31. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Buletin Oficial de Proprietate Intelectuală, nr.12, Chişinău, 2014, p.29-30.

29. Toderaş I., Cebotari V., Gulea A., Buzu I., Floquet S., Cadot E. Procedeu de obţinere a compusului [tetra-oxoetilendiaminotetraacetatdimolibden V] de bis-(tetrafenilfosfoniu) di-semihidrat şi procedeu de hrănire a albinelor cu utilizarea acestuia. Brevet de invenţie nr. MD 4438 B1 2016.10.31. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Buletin Oficial de Proprietate Intelectuală, nr. 10, Chişinău, 2016, p. 22-23.

30. Toderaş I., Cebotari V., Ungureanu L., Buzu I., Gheorghiţă C. Procedeu de hrănire a familiilor de albine *Apis mellifera.* Brevet de invenţie nr. MD 1061 Y 2016.08.31. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Buletin Oficial de Proprietate Intelectuală, nr.8, Chişinău, 2016, p. 33-34.

31. Toderaş I., Cebotari V., Ungureanu L., Buzu I., Gheorghiţă C. Procedeu de hrănire a familiilor de albine *Apis mellifera.* Brevet de invenţie nr. MD 1062 Y 2016.08.31. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Buletin Oficial de Proprietate Intelectuală, nr.8, Chişinău, 2016, p. 34.

32. Toderaş I., Cebotari V., Ungureanu L., Buzu I., Gheorghiţă C. Procedeu de hrănire a familiilor de albine *Apis mellifera.* Brevet de invenţie nr. MD 1079 Y 2016.10.31. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Buletin Oficial de Proprietate Intelectuală, nr.10, Chişinău, 2016, p. 29.

33. Ungureanu L., Gheorghiţă C. Tulpina microalgei verzi *Scenedesmus Apiculatus Hortob.* CLHE-H2. Agenţia de Stat pentru Proprietatea Intelectuală a Republicii Moldova. Depozit nr. a 2015 0027 din 18.03.2015. Chişinău, 2015.

34. Вассер С.П., Кондратьева Н.В., Масюк Н.П. и др. Водоросли: Справочник. Киев, «Наукова думка», 1989, 608 с.

35. Кожухарь И.Ф., Борш З.Т. Влияние карбонатной системы среды на культивирование водорослей при применении гидрокарбоната, как источника углеводного питания. Биологические ресурсы водоемов Молдавии. Кишинев, 1971, вып. 8, с. 8-4

36. Кузин В.С., Овчаренко Э.В., Алфимцев Н.А., Алфимцева Г.М. Способ стимуляции продуктивности и плодовитости пчел. Патент изобретения RU 2 199 210 C2 2002.09.20, Калуга, 2003.

37. Панин А.Н., Мелник Н.И., Малик Е.В., Вершинина И.Ю. Биопрепарат для повышения продуктивности пчел. Патент изобретения RU 2 166 322 C2, Москва, 2001.

38. Патент изобретения RU 2 380 406 C2 2010.01.27.

39. Плохинский Н.А. Руководство по биометрии для зоотехников. Изд. «Колос», Москва, 1989, 256 с.

40. Таранов Г.Ф. Корма и кормление пчел. Москва, Сульхозиздат, 1986, 158 с.

41. Яковлев А. С. Итоги исследований по влиянию стимулирующих подкормок на семьи пчел. Труды НИИ пчеловодства, вып. 7. Москва, Изд. «Московский рабочий», 1972, с. 87-101.