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TESTING OF THE NUTRIENT SUPPLEMENT ENRICHED WITH BIOMASS OF AQUATIC ALGAE IN THE BEE'S FEED

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Abstract

The purpose of this study was to test in the bee's feed the biomass of aquatic microalgae Scenedesmus quadricauda, hereinafter referred to as bioactive supplement "Scenecuadri" and elaboration on its basis of a process of feeding of bee families during the end of winter and start of spring (february-march), poor harvesting period in nature. The researchers was conducted on the Apis mellifera Carpatica bee families at the experimental apiary of the Institute of Zoology of the Academy of Sciences. For testing of biomass in bee's feed at the end of February, they were formed three batches of bees families, to which once for each frame with bees were administered 200 g of nutritional paste, prepared by mixing the powdered sugar with honey in proportion 7:3. The batch I - control, bees have received only nutritional paste, prepared by mixing the powdered sugar with honey. The batch II - the bees have received paste enriched with nutritional supplement "Apispir + Cr" in quantity of 200 mg of active substance per 1 kg of paste. The batch III - bees have received nutritional paste enriched with bioactive supplement "Scenecuadri" in a quantity equivalent to 200 mg of dry substance per 1 kg of paste. Research results have shown that feeding of bee families with nutritional supplement enriched with biomass of aquatic microalgae S. quadricauda help to increase, compared to the control batch, queen prolificacy up to 125 eggs or 7.8%, the amount of capped brood with 14.7 hundreds cell or 7.7%, family power by 0.30 kg or 9.3%, the amount of bee bread accumulated in nest with 14.0 hundreds cells or 15.5%, the amount of wax increased by 0.04 kg or 13.3%, resistance to disease by 1.6 or 1.8%, brood viability with 11 points or 1.2% and the amount of honey in the harvesting 3.01 kg, or 27.6%. The result is due to increasing nutrient assimilation and accessibility of biomass, given the fact that the microalgae Scenedsmus quadricauda is covered with a thin protective membrane and the biomass is rich in biologically active substances, in particular proteins, carbohydrates, lipids, essential amino acids, micro - and macro elements, antioxidants (beta carotene), which have a catalytic role in the metabolism of substances nitrogenous to worker bees, participates in the synthesis of enzymes, improves the qualitative composition of royal jelly and stimulates its secretion by wet nurses-bees, so indirectly influence (by feeding with royal jelly of the queen) on the reproductive system of the queen, intensifiing the ovogenesis and eggs laying. All of this largely determines the queen prolificacy, development of the larvae and brood from the nest, contributing to the increased family strength and their productive potential as a whole.

Key words: *bees, nutritional supplement, biomass, microalgae*, *Scenedesmus quadricauda*.

INTRODUCTION

At the end of winter (February) and early spring (March) reserves of natural food in the nest of bee family is exhausted and the deficiency of bioactive nutrients in the body of bees appears, especially of carbohydrates, protein, micronutrients, vitamins which have a decisive role in the physiological processes of vital activity of the bees organism, determining the reproduction and further development of the bee family on the whole (Cebotari et al., 2012; Cebotari et al. 2013a and 2013b; Toderaş et al., 2014).

In order to compensate the deficiency of nutritive substances in bees feeding during critical periods of harvesting in nature, most of the beekeepers fed the bees familie with sugar syrup, in the composition of which a number of important biologically active substances, excluding of carbohydrates, are absent. In these circumstances, identifying of available sources of biologically active substances for enriching the nutritional supplements from alimentar ration of bees in periods of poor harvest in nature, becomes an actual problem.

In recent decades researchers microbiologists have drawn attention to the biomass of mono- or multicellular microalgae, as important sources of biologically active substances. Among these the most studied became microalgae *Chlorella vulgaris* and multicellular microalgae (cyanobacteria) *Spirulina platensis* (Кожухарь et al., 1971; Baccep et al., 1989; Ionov et al., 2003; Mazo et al., 2004; Rudic et al., 2008a; Luca, 2012). Research has shown that biomass of microalgae *Ch. vulgaris* contains an important set of biologically active substances. According to certain authors (Luca, 2012), *Chlorella* is surnamed the "*supplement of energy and vitality*", having therapeutic properties, improving the health of the the organism in general and in particular fortifying the immune system, increases body resistance against infections. This microalgae is rich in β-carotene and is able to remove pesticide residues from the body, ingested through food, extract deposits of mercury, and is therefore a powerful detoxifier. Testing of suspension of biomass

of microalgae in in the feeding of the bees has helped to increase the rhythm of development of the colonies with 17.0-22.4% (Eremia et al., 2013).

Among the multitude of species of algae, the most studied is *S. platensis* (Rudic et al., 2006b; Rudic et al., 2006a; Bulimaga et al., 2006a and 2006b; Rudic et al., 2007; Rudic et al., 2008a and 2008b). For over 20 years this multicellular filamentous cyanophytes microalga has been explored as a food source. The World Health Organization and the 3rd International Congress of Food Science and Technology unconventional defined Spirulina as an essential source of up to 50 bioactive substances, which ensures the normal vital processes of the human and animal body.

To strengthen the vigor and disease resistance of bee families, some experts have proposed to enrich the nutritional supplements with biomass of strain *Bifidocbacterium globosum* + biomass of *Streptococcus faecium* + carbohydrate + oxide and aluminum hydroxide + ascorbic acid (Панин et al., 2001), suspension the *Karnitinhlorid* (Кузин et al., 2003).

In beekeeping are known also other proceeding to stimulate growth of bee families by feeding with sources of biologically active substances, in particular sugar syrup mixture of 50% enriched with biomass microalgae *S. platensis (Nordst.) Geitl CALU-835* (Toderaş et al., 2003). The disadvantage of this proceeding is the low efficiency, because the cells of that cyanophytes microalgae are covered with relatively thick a protective membrane, which stagnates the digestion process of nutrients from biomass by bees, in addition, the sugar syrup can not be used in winter when air temperatures are low.

Among the known proceedings (Toderaş et al., 2012, 2012b and 2012c), the nearest solution after technical essence and the obtained result is the proceeding of feeding bee families *Apis mellifera* MD 476Z 2012.09.30. (Toderaş et al., 2012b), which includes the feeding of bees in spring with a mixture of solution of 1% mas. biomass extract of microalgae *S. platensis* CNM-CB-02 and 50% sugar syrup in the ratio of 1: 500, respectively. Previously, to obtain biomass of *S. platensis,* microalgae has been cultivated in the presence of coordinative organic compound of chromium and potassium alum - $KCr(SO_4)_2 12H_2O$, which increases the permeability of living cells. The feeding of the bees with this mixture was carried out every 2 days, for two weeks in an amount of 100 ... 130 ml of a mixture on bee frame.

The disadvantage of this proceeding is the fact that the technology of obtaining of the extract biomass of microalgae *S. platensis* cultivated in the presence of coordinative compound is too complicated and expensive, and the mixture of sugar syrup enriched with supplement of bioactive substances can not be used during winter or early spring, because of high humidity created by a large amount of releasing vapor in the nest during the period when bees are in hibernation skein. For these reasons, some researchers have proposed as a source of biologically active substances biomass aquatic microalgae (Ungureanu et al., 2015), which are more accessible and less expensive.

In this context, the aim of this paper was to test in feeding of bees biomass of aquatic microalgae *Scenedesmus quadricauda* and elaboration on its basis of a proceeding for feeding of bee families during the end of winter and start of spring (february-march) poor harvesting period in nature.

MATERIALS AND METHODS

The researches were conducted on the *Apis mellifera Carpatica* bee families at the experimental apiary of the Institute of Zoology of the Academy of Sciences To achieve the purpose, experimental plan has been made that included the feeding of bee families at the end of winter during poor harvesting in nature, when atmospheric temperatures were low, with the nutrition paste wich was prepared by mixing powdered sugar with honey in proportion 7:3 and bioactive supplements. As a bioactive supplement the biomass of aquatic microalgae *S. quadricauda* was used, that was mixed with the pasta. The feeding of the bee families with enriched paste was performed by its distribution in the form of expellers in the nest, above the frame. Usually one expeller for every frame with bees was put.

Bioactive supplement, called by us "*Scenecuadri*", represents a suspension of biomass of 2%, greenish yellow coloured, dry matter contains 47-49% proteins, which includes the complete set of essential and non-essential amino acids, 40-46 % of carbohydrate, 11.9 to 12.2% of lipids, vitamins, micro- and macro elements and other important bioactive substances. In 100 mg of dry matter of the supplement there is 0.28 to 0.31 mg of beta-carotene, one of the main components with antioxidant properties and catalyzation of regeneration processes of cells and reproductive tissues of the queen and function lactogenic at the bee working. Considering that the monocellular microalgae Scenedesmus quadricauda is covered by a relatively thin protective membrane, bioactive substances from biomass are available for digestion in the digestive tract of honey bees.

To estimate the efficiency the proceeding of the bees feeding with the supplement, at the end of february were initiated experiments of comparative testing of the paste on bee colonies divided into three batches, 13 to 15 families each batch. The supplement was administered once, 200 g nutritional paste (a cake) for every frame with bees. The batch I - control, bees have received only nutritional paste, prepared by mixing the powdered sugar with honey in previously mentioned proportion. The batch II – the closest solution, the bees have received paste enriched with nutritional supplement “Apispir + Cr" ” (Toderaş et al., 2012b) in quantity of 2 ml solution with a concentration of 10% (200 mg of active substance) per 1 kg of paste. The batch III - the proposed invention, bees have received nutritional paste enriched with bioactive supplement "*Scenecuadri*" in a quantity 10 ml of suspension with a concentration of 2.0% (200 mg of dry substance) per 1 kg of paste.

In 100 days after feeding bees with the nutritional bioactive supplement (which coincided with the first

harvest) principal morph-productive characters of reproduction, development and productivity of bee families in the experimental batches were evaluated, according to Zootechnical norme regarding breeding of bee families, the growth and certification of genitor beekeeping material, approved by Government Decision no. 306 of 28.04.2011 (Zootechnical norme, 2011). The obtained in experience data were statistically processed using computer software "STATISTICA - 6" and evaluated their certainty, according to variation biometric statistics, by methods of Плохинский, 1989.

RESULTS AND DISCUSSIONS

The test results showed that the feeding of bees with the supplement "*Scenecuadri*" at the end of winter during poor harvest in nature, has contributed to a significant increase of value of principale morpho-productive characters. (Tab. 1).

It was found that the biologically active substances in the supplement "*Scenecuadri*" indirectly have caused stimulation of reproductive functions of the queen (oogenesis) contributing to the growth of egg-laying and to the increasing of the capped brood in the nest and as a result, the development of the bee family. Because, the queen does not consume nutritional supplement administered in the nest, but is constantly fed by worker bees with royal jelly, we can say that the biologically active substances in the supplement have a stimulating impact on lactogenic functions of the nurses bee and on the qualitative composition of royal jelly, stimulating, thus, reproductive functions of the queen.

As a result, the queen's prolificacy in hives from the batch III increased significantly compared with those in batch I (control) and batch II (the nearest solution), respectively, with 125 and 100 eggs/24 hours, or 7.8 and 6.2% (td = 3.5 and 2.8; P<0,001 and P<0.01).

Also, the quantity of capped brood at the hives from the batch III was significantly higher compared to batch I and batch II, 14.7 and 12.0 hundred cells, or 7.7 to 6.2% (td = 3.4 and 2.7, P<0.001 and P<0.01), respectively.

The increasing of the queens prolificacy and amount of capped brood, indirectly led to a significant increase of family strength, expressed by the total population of bees in the nest. Thus, the power of bee families from the batch III was significantly higher compared to group I and group II (the nearest solution), with 0.30 and 0.27 kg of bee, or 9.3 and 8.3% (td = 3.5 and 3.4; P < 0.001), respectively.

Due to higher family strength, the colonies from experimental batches II and III have obtained more pronounced feature of accumulation of the final bee products in the nest, for which, actually, are bred and exploited the bees.

The quantity of bee bread accumulated in the nest was also positively influenced by nutritional supplements enriched with algal biomass. Thus bee families from experimental batches II and III, who received nutritional supplements enriched both with extract of biomass of *Apispir*+Cr and biomass of microalgae *S. quadricauda* significantly exceeded the families from the control batch after the quantity of bee bread accumulated in the nest, respectively, 7.6 and 14.0 hundred cells, or 8.4 to 15.5% (t_d=2.6 and 3.4; P<0.05 şi P<0.01). Also compared with batch II, the amount of accumulated bee bread in the nest at the bee families in group I, who received in food nutritional supplement enriched with biomass of *Scenecuadri*, only had a tendency to increase by 6.6% (t_d=1.6; P<0.1).

The amount of wax accumulated during this period in the nest was also, positively influenced by the nutritional supplement enriched with biomass of microalgae *S. quadricauda*. Thus, bees families in the experimental batch III significantly exceeded the families from the batch after amount of wax accumulated at the first harvesting with 0.04 kg or 13.3% (t_d=2.9; P<0.01) and families from batch II with 0.03 kg or 9.7% (t_d=2.1; P<0.05).

Finally, the amount of honey accumulated in the nest, the morpho-productive character with important economic value, was also the most positively influenced by the biologically active substances contained in supplements enriched with both extract of biomass of *Apispir* + Cr and bioactiv suppliment *Scenecuadri*. Thus after the amount of honey collected at the first harvesting, the bees families from experimental batches II and III vastly exceeded families in the control batch I with 1.03 and 3.01 kg, or 9.6 and 27.6% (t_d=2.0 and 7.2; P<0.05 şi P<0.001). The data presented in table reveals that bees families from the batch III had greatest capacity of accumulation of products in the nest, and after honey production, significantly exceeded the families from the experimental group II, with 1.98 kg or 16.8 %. This difference is veridical with the highest threshold of certainty without error probability forecasts after Stiudent (t_d = 4.5; P<0.001).

More obvious influence of biologically active substances from the extract or biomass of microalgae on the morpho-productive characters of bee families is presented in histogram 1.

In the histogram it can be seen that the characters of accumulation in the nest of bee products such as bee bread, wax and honey had the greatest growth rates. In particular the quantity of honey had rose the most obvious at the bee families who received by food biologically active substances from supplements enriched with biomass of aquatic microalgae.

However, we have to mention that biologically active substances from biomass of microalgae have had a beneficial influence on disease resistance features and viability of the brood.

So the brood viability of bee families of batches II and III, who received supplements enriched, respectively, with extract of biomass Apispir + Cr and biomass Scenecuadri, was significantly higher compared with controls, with 1.3 to 1.2% (t_d=3.1 and 2.2; P<0.01 şi P<0.05). Given the fact that biological variability of this feature is very narrow, the

significance of this difference (small at first sight, as the absolute dimension) is quite high and corresponds

Table 1. The test results of feeding of bees families with nutritional supplements fortified with algal biomass

Batch and name of bioactive substances	Nr. of bee fam.	The value of the character at first harvesting, M ± m	The difference compared to batch I (control)			The difference compared to batch II (the nearest solution)		
			d	%	t_d	d	%	t_d
Prolificacy of queen, eggs/24 hours								
I control	14	1593 ± 25	-	-	-	-25	1.6	0.7
II Apispir+Cr	15	1618 ± 26	+ 25	1.6	0.7	-	-	-
III Scenecuadri	13	1718 ± 25	+ 125**	7.8	3.5	+100**	6.2	2.8
Quantity of capped brood, hundred cells								
I control	14	191.5 ± 3.1	-	-	-	-2.7	1.4	0.6
II Apispir+Cr	15	194.2 ± 3.2	+ 2.7	1.4	0.6	-	-	-
III Scenecuadri	13	206.2 ± 3.0	+ 14.7**	7.7	3.4	+12**	6.2	2.7
Family strength, kg								
I control	14	3.22 ± 0.05	-	-	-	-0.03	0.9	0.5
II Apispir+Cr	15	3.25 ± 0.04	+ 0.03	0.9	0.5	-	-	-
III Scenecuadri	13	3.52 ± 0.07	+ 0.30**	9.3	3.5	+0.27**	8.3	3.4
Quantity of bee bread, hundreds of cells								
I control	14	90.1 ± 2.1	-	-	-	-7.6*	8.4	2.6
II Apispir+Cr	15	97.7 ± 2.1	+ 7.6*	8.4	2.6	-	-	-
III Scenecuadri	13	104.1 ± 3.6	+ 14.0**	15.5	3.4	+6.4	6.6	1.5
Quantity of honey, kg								
I control	14	10.74 ± 0.35	-	-	-	-1.03*	9.6	2.0
II Apispir+Cr	15	11.77 ± 0.37	+ 1.03*	9.6	2.0	-	-	-
III Scenecuadri	13	13.75 ± 0.24	+ 3.01***	27.6	7.2	+1.98***	16.8	4.5
Quantity of wax, kg								
I control	14	0.30 ± 0.01	-	-	-	-0.01	3.3	0.7
II Apispir+Cr	15	0.31 ± 0.01	+ 0.01	3.3	0.7	-	-	-
III Scenecuadri	13	0.34 ± 0.01	+ 0.04**	13.3	2.9	+0.03*	9.7	2.1
Resistance to disease, %								
I control	14	88.9 ± 0.8	-	-	-	-1.5	1.7	1.6
II Apispir+Cr	15	90.4 ± 0.5	+ 1.5	1.7	1.6	-	-	-
III Scenecuadri	13	90.5 ± 0.6	+ 1.6	1.8	1.6	+0.01	0.1	0.01
Broods viability, %								
I control	14	90.0 ± 0.3	-	-	-	-1.1**	1.2	3.1
II Apispir+Cr	15	91.1 ± 0.2	1.1**	1.2	3.1	-	-	-
III Scenecuadri	13	91.1 ± 0.4	1.1*	1.2	2.2	0.0	0.0	0.0

Remark: * P<0.05; ** P<0.01; *** P<0.001;

to a high threshold of certainty, according to probability theory predicts without error Student [Плохинский, 1989].

Similarly, bees families of these experimental batches had a tendency to have increased resistance to the diseases. Despite the fact that the biologically features like brood viability and disease resistance are largely determined by heredity (h2 = 0.7-0.8), however, the test data demonstrates that feeding of bees with biologically active substances certainly have contributed to fortify immunity and strength of bee families, as the result - to the increase of their productivity.

Therefore, the technical result of the use of biomass of microalgae in the nutrition of bees consist in stimulate functions of ovogenesis and egg laying at queens, increasing the quantity of capped brood and of the number of hatched worker bees, which in its turn, led to the quantitative increasing of the power of bee families, the harbinger of higher productivity. The result is due to increasing nutrient assimilation and accessibility of biomass, given the fact that the micro S. quadricauda is covered with a thin protective membrane and the biomass is rich in biologically active substances, in particular proteins, carbohydrates, lipids, essential amino acids, micro and macro elements, antioxidants (beta carotene), which have a catalytic role in the metabolism of substances nitrogenous to worker bees, participates in the synthesis of enzymes, improves the qualitative composition of royal jelly and stimulates its secretion by nurses bees, so indirectly influence (by feeding with royal jelly of the queen) on the reproductive system of the queen, intensifiing the ovogenesis and laying eggs.

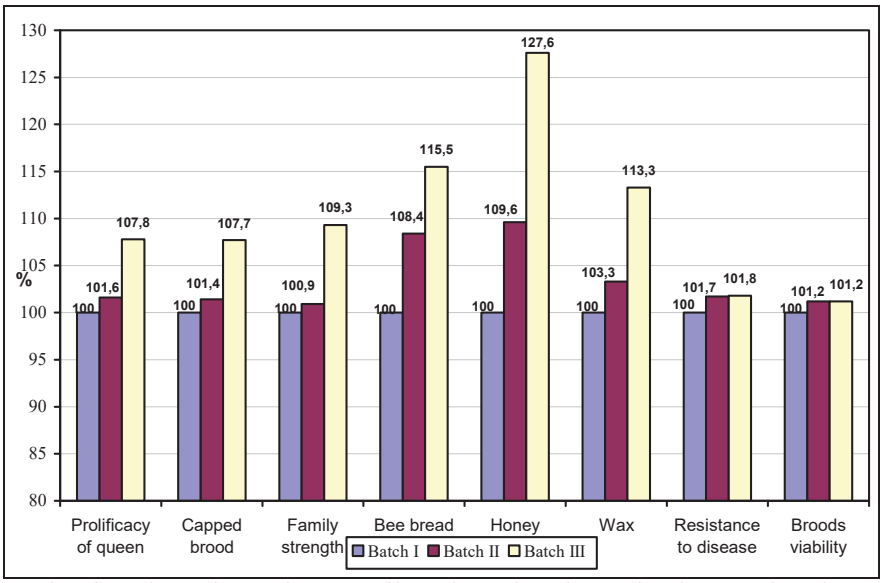

Fig. 1. Value of morpho-productive characters of bee colonies depending on the administered supplement

All of this determines largely prolificacy of the queen, development of larvae and brood in the nest, contributing to increased strength of bee families and their productive potential entirely.

On the base the carried investigations it was elaborated a new proceeding of feeding of bee families in poor harvesting period in nature, at the end of winter - early spring (February-March), when atmospheric temperatures are low. The proceeding provides the enrichment of the nutritional supplements with biologically active substances, which are a little cheaper and easier to obtain, more accessible and more easily digested by bees, ensuring at the same time, the nutritional needs of bees during this period of year. Biologically active substances, added to the food, stimulates prolificacy of bees queen, increase the power and productivity of bee families of *Apis mellifera.*

CONCLUSIONS

1. The feeding of bee families with nutritional supplement enriched with biomass of aquatic microalgae *S. quadricauda* help to increase, compared to the control batch, the queen prolificacy with 7.8% ($P<0.01$), the amount of capped brood with 7.7% ($P<0.01$), family power with 9.3% ($P<0.01$), the amount of bee bread accumulated in nest with 15.5% ($P<0.01$), the amount of wax increased with 13.3% ($P<0.01$), resistance to disease with 1.8% ($P<0.1$), brood viability with 1.2% ($P<0.05$) and the amount of honey in the harvestig with 27.6% ($P<0.001$).

2. On the base the carried investigations it was elaborated a new proceeding of feeding of bee families in poor harvesting period in nature, when atmospheric temperatures are low. This proceeding ensuring the increase of productivity of bee families.

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