NEW NUTRITIONAL SUPPLEMENTS FOR BEES DURING DEFICIENT HARVESTING PERIODS

Valentina Cebotari[1*], I. Buzu[1], Olesea Gliga[1], Olga Postolachi[1]

[1]*Institute of Zoology of the Academy of Sciences of Moldova, Chişinău, Republic of Moldova*

Abstract

The purpose of this study was to test in the bee's feed the biomass of aquatic microalgae Oocistis borgei Snow, hereinafter referred to as bioactive supplement „Borgesnow" and elaboration on its basis of a process of feeding of bee families during the end of winter and start of spring (february-march), deficient harvesting period in nature. The researchers were 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+Fe" 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 "Borgesnow" 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 Oocistis borgei Snow help to increase, compared to the control batch, queen prolificacy up to 132 eggs or 8.3%, the amount of capped brood with 15.4 hundreds cell or 8.0%, family power by 0.26 kg or 8,1%, the amount of bee bread accumulated in nest with 20.4 hundreds cells or 22,6%, the amount of wax increased by 0.09 kg or 30,0%, resistance to disease by 3.1 points or 3,5%, brood viability with 1.5 points or 1,7% and the amount of honey in the harvesting 4.16 kg, or 38,7%. The result is due to increasing nutrient assimilation and accessibility of biomass, given the fact that the microalgae Oocistis borgei Snow 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, which have a catalytic role in the metabolism of nitrogenous substances to worker bees, participates in the synthesis of some enzymes, improves the qualitative composition of royal jelly and stimulates its secretion by nurse-bees, so indirectly influence (by feeding with royal jelly of the queen) on the reproductive system of the queen, intensifiing the ovogenesis and egg-laying. All of these 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, Oocistis borgei Snow

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 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 [3, 4, 5, 18].

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

*Corresponding author: valentinaceb@yahoo.com
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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* [7, 8, 9, 13, 23, 24]. Research has shown that biomass of microalgae *Ch. vulgaris* contains an important set of biologically active substances. According to certain authors [8], *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 therefore is a powerful detoxifier. Testing of suspension of microalgae's biomass in the feeding of the bees has helped to increase the rhythm of development of the colonies with 17.0-22.4% [6].

Among the multitude of species of algae, the most studied is *S. platensis* [1, 2, 11, 12, 13, 14, 15]. 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 fortify 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 [25], suspension of karnitine chlorid [24].

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 [17]. The disadvantage of this proceeding is the low efficiency, because the cells of that cyanophytes microalgae are covered with relatively thick 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 [18, 19, 20], the nearest solution after technical essence and the obtained result, is the proceeding of feeding bee families *Apis mellifera* MD 476Z 2012.09.30. [20]. This process involves feeding the bees in spring with a mixed solution of 1% mas. biomass of microalgae Spirulina platensis extract CNM-CB-02 and 50% sugar syrup in the ratio of 1: 500. Previously, to obtain biomass of *S. platensis*, the microalgae has been cultivated in the presence of coordinative organic compound of *ferric Fe (III) selenite hexahydrate* ($FeSeO_3 \cdot 6H_2O$), 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 biomass extract 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 claster. For these reasons, some researchers have proposed as a source of biologically active substances biomass of aquatic microalgae [21], 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 *Oocistis borgei Snow* 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 deficient 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 *Oocistis borgei Snow* 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 candy in the nest, above the frame. Usually one candy for every frame with bees has been used.

Bioactive supplement, called by us "*Borgesnow*", represents a suspension of biomass of 2%, greenish yellow coloured, dry matter contains 23-25% proteins, which includes the complete set of essential and non-essential amino acids, 30-45% of carbohydrate, 8.1-9.2% of lipids, vitamins, micro- and macro elements and other important bioactive substances.

In 100 mg of dry matter, the supplement contains 7.5-8.0% of immunogenic amino acids and 15.1-16.0% proteinogenic amino acids, one of the main components with antioxidant properties and catalyzation of regeneration processes of cells and reproductive tissues of the queen and lactogenic at the bee working. Considering that the monocellular microalgae *Oocistis borgei Snow* 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 "*Borgesnow*", at the end of february were initiated experiments of its comparative testing on bee colonies, divided into three batches, 13 to 15 families each batch. The supplement was administered once, 200 g nutritional paste (a candy) for every frame with bees. The batch I - control, bees have received only nutritional paste, prepared by mixing the powdered sugar with honey in proportion 7:3. The batch II – the closest solution, the bees have received paste enriched with nutritional supplement "*Apispir+Fe*" [20] in quantity of 2 ml solution with a concentration of 10% (200 mg of active substance) per 1 kg of paste. The batch III - experimental, bees have received nutritional paste enriched with bioactive supplement "*Borgesnow*" 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 [10]. The obtained data were statistically processed using computer software "STATISTICA-6" and evaluated their certainty, according to biometric variational statistics, by methods of Плохинский, 1969 [26].

RESULTS AND DISCUSSIONS

The test results showed that the feeding of bees with the supplement "*Borgesnow*" at the end of winter during deficient 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 "*Borgesnow*" 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, to the development of the bee family.

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+Fe	15	1618 ± 26	+ 25	1.6	0.7	-	-	-
III Borgesnow	13	1725 ± 14	+ 132***	8.3	4.6	+108***	6.6	3.7
Quantity of capped brood. hundred cells								
I control	14	191.5 ± 3.1	-	-	-	-2.7	1.4	0.6
II Apispir+Fe	15	194.2 ± 3.2	+ 2.7	1.4	0.6	-	-	-
III Borgesnow	13	206.9 ± 1.6	+ 15.4***	8.0	4.4	+12.7***	6.5	3.5
Family strength. kg								
I control	14	3.22 ± 0.05	-	-	-	-0.03	0.9	0.5
II Apispir+Fe	15	3.25 ± 0.04	+ 0.03	0.9	0.5	-	-	-
III Borgesnow	13	3.48 ± 0.07	+ 0.26**	8.1	3.3	+0.23**	7.1	2.9
Quantity of bee bread. hundreds of cells								
I control	14	90.1 ± 2.1	-	-	-	-7.6*	8.4	2.6
II Apispir+Fe	15	97.7 ± 2.1	+ 7.6*	8.4	2.6	-	-	-
III Borgesnow	13	110.5 ± 1.5	+ 20.4***	22.6	7.9	+12.8***	13.1	5.0
Quantity of honey. kg								
I control	14	10.74 ± 0.35	-	-	-	-1.03*	9.6	2.0
II Apispir+Fe	15	11.77 ± 0.37	+ 1.03*	9.6	2.0	-	-	-
III Borgesnow	13	14.90 ± 0.25	+ 4.16***	38.7	9.7	+3.13***	26.6	7.0
Quantity of wax. kg								
I control	14	0.30 ± 0.01	-	-	-	-0.01	3.3	0.7
II Apispir+Fe	15	0.31 ± 0.01	+ 0.01	3.3	0.7	-	-	-
III Borgesnow	13	0.39 ± 0.01	+ 0.09***	30.0	6.4	+0.08***	25.8	5.7
Resistance to disease. %								
I control	14	88.9 ± 0.8	-	-	-	-1.5	1.7	1.6
II Apispir+Fe	15	90.4 ± 0.5	+ 1.5	1.7	1.6	-	-	-
III Borgesnow	13	92.0 ± 0.5	+ 3.1**	3.5	3.3	+1.6*	1.8	2.3
Broods viability. %								
I control	14	90.0 ± 0.3	-	-	-	-1.1**	1.2	3.1
II Apispir+Fe	15	91.1 ± 0.2	1.1**	1.2	3.1	-	-	-
III Borgesnow	13	91.5 ± 1.3	1.5	1.7	1.1	0.4	0.4	0.3

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 bee-nurse 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 132 and 108 eggs / 24 hours, or 8.3 and 6.6% (td=4.6 and 3.7; P<0.001 and P<0.001).

Also, the quantity of capped brood at the hives from the batch III was significantly higher compared to batch I and batch II, 5.4

and 12.7 hundred cells, or 8.0 to 6.5% (t_d = 4.4 and 3.5; 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, 0.26 and 0.23 kg of bee, or 8.1 and 7.1% (t_d =3.3 and 2.9; P < 0.001).

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+Fe* and biomass of microalgae *Oocistis borgei Snow* significantly exceeded the families from the control batch after the quantity of bee bread accumulated in the nest, respectively , 20.4 and 12.8 hundred cells, or 22.6 and 13.1% (t_d=7.9 and 5.0; P<0.05 and P<0.01).

The amount of wax, accumulated during this period in the nest, was also positively influenced by the nutritional supplement enriched with biomass of microalgae *Oocistis borgei Snow*. 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 0.09 kg or 30.0% (t_d=6.4; P<0.01) and families from batch II with 0.08 kg or 25.8% (t_d=5.7; P<0.05).

As a final result, 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+Fe* and bioactiv suppliment "*Borgesnow*". 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 4.16 kg, or 9.6 and 38.7% (t_d=2.0 and 9.7; P<0.05 and 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 3.13 kg or 26.6%. This difference is veridical with the highest threshold of certainty without error probability forecasts after Stiudent (t_d=7.0; 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 Fig.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 *Oocistis borgei Snow.*

Also 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+Fe* or "*Borgesnow*", was significantly higher compared with controls, with 1.5 to 3.1% (t_d=1.6 and 3.3; P<0.01 and P<0.01). 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 to a high threshold of certainty, according to probability theory of predictions, without error Student [26].

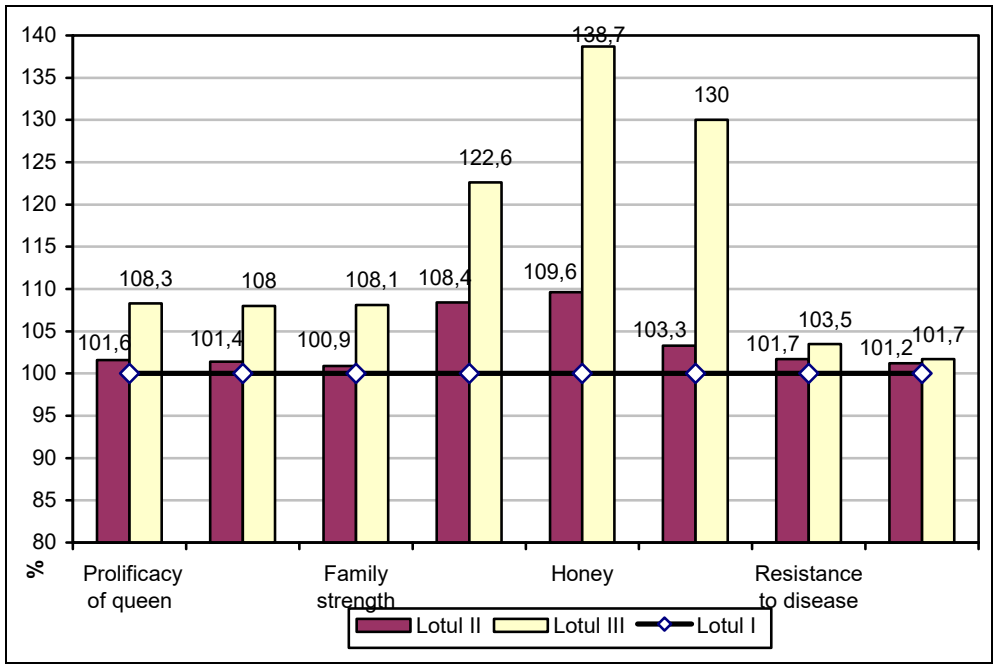

Fig. 1 Value of morpho-productive characters of bee colonies depending on the administered supplement

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 (h^2=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 *Oocistis borgei Snow* 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, which have a catalytic role in the metabolism of nitrogenous substances to worker bees, participates in the synthesis of enzymes, improves the qualitative composition of royal jelly and stimulates its secretion by nurse-bees, so indirectly influence (by feeding with royal jelly of the queen) on the reproductive system of the queen, intensifiing the ovogenesis and egg-laying.

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 deficient 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 *Oocistis borgei Snow* help to increase, compared to the control batch, the queen prolificacy with 8.3% ($P<0.001$), the amount of capped brood with 8.0% ($P<0.001$), family power with 8.1% ($P<0.01$), the amount of bee bread accumulated in nest with 22.6% ($P<0.01$), the amount of wax increased with 30.0% ($P<0.001$), resistance to disease with 3.5% ($P<0.1$), brood viability with 1.7% ($P<0.01$) and the amount of honey in the harvestig with 38.7% ($P<0.001$).

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

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