ENRICHING NUTRITIONAL SUPPLEMENTS FOR BEES WITH BIOLOGICALLY ACTIVE SUBSTANCES OF COORDINATIVE ORGANIC COMPOUNDS

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Abstract

The purpose of this study was comparative testing, in bee food, of various nutritional supplements enriched with some new organic coordinative compounds of new generation and to reveal the most effective ones for the preparation of nutritional supplements and bee nourishment during periods of poor harvesting in nature. The research was carried out on honey bee families from the Carpathian race at the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova. In the experiment, 5 similar batches were formed, with 10 families in each batch. The bee families of the experimental batches were fed for 14 days with nutritional supplements enriched with biologically active substances, a 50% of nutritional blend of sugar syrup and bioactive supplements of coordinative organic compounds (COC).The feeding of bee families with nutritional mixtures was carried out in ratio of 120 ml of the mixture at each interval of populated by bees frame, every 2 days for two consecutive weeks. In batch I (control), bee families were fed only with 50% sugar syrup. For bees of batch II (prototype), the sugar syrup was enriched with the patented bioactive suppliment (MD 850 Z 2015.08.31). For bee families of batches III, IV and V, nutritional supplements enriched with coordinative organic compounds containing rare microelements, referred to as CV-6, CV-8 and CV-22, were used. The research results have shown that some of the coordinative organic compounds tested in bee's feed in the period of poor harvesting in nature, had some influence on reproductive and characteristics of bee families. Thus, in batches II, III and V, compared to the control group, the queen prolificity was higher by 9.9-15.8% ($P<0.05 – 0.001$), amount of capped brood by 10,0-15,8% ($P<0.05 – 0.001$) and colony strength by 5,8-6,2% ($P<0.001$).However, the stimulating effect of reproductive functions is not certain, because the activation of reproductive and developing characters of bee families must inevitably lead to the increasing of the capacity of acumulation of bee products in the nest. Therefore, none of the newly-tested coordinating compounds showed general stimulatory properties, which would result in increased apiculture production, especially honey production.

Key words: testing, nutritional supplements, bee families, prolificity, development, honey

INTRODUCTION

The problem of maintaining balanced nutrition of bee families in periods of poor harvesting in nature has been and is constantly in the attention of beekeepers and field specialist as well as scientific researchers, who develop scientific bases to ensure the nutrition of bees in different times of the year. Particularly, the issue of balanced bee nutrition is increasing during the early spring (March-April), when the bee organism suffers from a deficiency of biologically active substances, especially micro-macro elements, which play a crucial role in catalysing of physiological processes of living organisms [17], including bees, as well as the development of bee colonies as a whole. In order to balance the bee's nutrition in the poor harvesting period in nature, beekeepers nourish bee families with nutritional supplements prepared mainly from sugar syrup mixed with various protein, vitamin and mineral ingredients. As proteic ingredients are used more frequently pollen and skimmed cow milk [18, 40, 41], soybean or sunflower grist [3-5] and, less frequently, aminic acids [36], yeasts [18] and are used. At the same time, if the spring is early and warm, the honey bees fully ensure the need for protein

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nutrition from nature pollen. In this case, beekeepers recommend complementing of the carbohydrate nutritional supplements with biologically active substances containing vitamins and microelements [4, 5, 18, 36, 40, 41].

Some authors have demonstrated that the use in bees feed of $CoCl_2$ and $MnSO_4$ native salts, mixed with sugar syrup, has contributed to the increase in the amount of brood and the production of honey accumulated in the nest [41]. Other researchers reports that increasing the bee families development and their productivity can be achieved by adding a quantity of *Tazlau salts* to the carbohydrate nutritional supplement in mixture with *sodium* and *potassium chloride*[20]. In other information [22] communicates using the feeding of bees of a mineral premix (pellets tiny phosphate glass) with a high content of macro trace elements (Fe, Mn, Ca, Mg, Cu, Zn, I, S, Co, Mo, Se), which essentially reduce the winter mortality of honey bees and increase their productivity yields during harvesting.

However, according to the practical experience of beekeepers, the use of native mineral salts as a source of micro-macro elements for bee nutrition mostly has resulted in no remarkable successes, since these (native salts) are not sufficiently assimilated into the bee's digestive tract. For these reasons, in the last decades, researches have been activated to reveal sources of natural biologically active substances both from sources of plant origin [34] and animal sources [6, 11, 12, 26, 27, 28 , 29]. Thus, a number of investigations [2, 35, 37] have shown that the biomass of microalga *Chlorella vulgaris* contains an important set of biologically active substances, so it can be called the "*energy and vitality supplement*" with therapeutic properties, improving the state of body health in general, and fortifying the immune system, in particular, increases the body's resistance against infections. This microalgae, rich in β-carotene, is able to remove residuals and pesticides from the body, ingested from food, extract mercury deposits, thus being a powerful detoxifying agent. Some scientists [16] report that feeding of bee families with the *Chlorella vulgaris* biomass suspension contributes to the increasing of the acceptance degree of transferred larvae for queens breeding, to the increase the size of the queen larvae and, as a result, to queens with increased body mass.

Another important source of biologically active substances is biomass or biomass extract of filamentous microalgae (cyanobacteria) *Spirulina platensis*. Our previous research [11-13, 29, 31-33], carried out in collaboration with the researchers of the Institute of Microbiology and Biotechnology of the ASM and those from the State University of Moldova, showed that the use in bee nourishment, during periods of poor harvesting in nature, of nutritional supplements enriched with *Spirulina platensis* cyanobacterium biomass extract, grown in the presence of coordinative organic compounds containing rare microelements, contributed to the significant increase in the prolificity of the queen, the amount of capped brood, the viability of the brood, the resistance to disease of the bee families, the amount of waxes grown in the nest, the amount of bee bread and honey accumulated in the nest. Based on these researches, new generation of bioactive organic remedies have been elaborate, which allow conventional beekeeping to be converted to organic beekeeping.

Aquatic microalgae also present another important source of biologically active substances to enrich nutritional supplements for bees. Our researches [6-8, 24, 26-28] has shown that the use in bees feed during the poore harvesting period in natere of nutritional supplements enriched with *Scenedesmus quadricauda* aquatic microalgae, *Oocystis borgei Snow*, *Scenedesmus apiculatus*, ensures significant increase in the prolificity of the queens, the quantity of capped brood, the strength of the family, the viability of the brood, the resistance of the bee families to the diseases, the amount of the accumulated wax, the amount of bee bread and honey accumulated in the nest. At the same time, we mention that the cultivation of *Spirulina platensis* and the above-mentioned aquatic microalgaes

requires rather complicated and costly biotechnologies, which are accessible only to institutions, equipped with special tools and equipments, as well as well-trained specialists in the field.

Therefore, the researchers of the Zoology Institute in collaboration with the State University of Moldova, as well as those from the Versailles Lavoisier Institute, France, undertook research to reveal coordinative organic compounds (COC) containing biological active substances that could be applied to bees feed. Due to the simpler and less costly production technology, these coordinating organic compounds become more accessible. Beacouse of their chemical structure and the constituent bioelements (complex and bipolar ions with variable valences, organic ligands of different origins), as well as their molar ratio of combinations, the coordinating organic compounds possess a wide spectrum of bioactive properties [15]. They are used in various biotechnologies to synthesize and produce a range of pharmaceutical preparations, such as: vitamin B12 and hemoglobin [1], antidote and detoxifying [38], antibacterial [23] and antifungal [21] properties

Our previous research [9, 10, 25, 30] has shown that feeding bee families during periods of poor harvesting in nature with nutritional supplements, enriched with some coordinative organic compounds, containing rare microelements, has contributed to a significant increase in the prolificity of the queens, the amount of capped brood, the strength of the family, the viability of the brood, the resistance of the bee families to the diseases, the amount of accumulated wax, the amount of bee bread and honey accumulated in the nest.

At the same time, considering that coordinative organic compounds are synthesized by several scientists, in enormous varieties of structures with different chemical formulas, these (compounds) also have a very wide range of bioactive properties that are not yet far away sufficient studied.

In this context, the purpose of our research was to test the various nutritional supplements enriched with some new organic coordinative compounds of new generation in bees' food, and to reveal the most effective ones for the preparation of nutritional supplements and bee nourishment during periods of poor harvesting in nature.

MATERIALS AND METHODS

The research was carried out on honey bee families from the Carpathian race at the experimental apiary of the Institute of Zoology of the Academy of Sciences of Moldova, located at the stationary in Ghidighici Silvic District, Canton no. 8.

For testing of supplements, enriched with biologically active substances of coordinative organic compounds, in bee nutrition, an experiment was carried out at the beginning of April (shortfall in nature) according to the following scheme (Tab. 1).

Table 1 Scheme of the experiment of testing in bee feed of nutritional supplements enriched with bioactive substances of COC

Batch	The bee family livestock	Name of nutritional supplement and symbolic formula of bioactive substances	Dose of bioactive substance of supplement / 1 L of syrup	Specification
I	10	Control, 50% sugar syrup	-	Control
II	10	Syrup + BT-2, Co+BiHcdta•5H_2O (Co+Bi)	0.02 mg/L	Prototype
III	10	Syrup + CV-6	0.02 mg/L	Experiment
IV	10	Syrup + CV-8	0.02 mg/L	Experiment
V	10	Syrup + CV-22	0.02 mg/L	Experiment

In the experiment, 5 similar batches were formed, with 10 families in each batch. The bee families of the experimental batches were fed for 14 days with nutritional supplements enriched with biologically active substances, a 50% of nutritional blend of sugar syrup and bioactive supplements of coordinative organic compounds. The feeding of bee families with nutritional mixtures was carried out in ratio of 120 ml of the mixture at each interval of populated by bees frame, every 2 days for two consecutive weeks.

In batch I (control), bee families were fed only with 50% sugar syrup.

For bees of batch II (prototype), the sugar syrup was enriched with the patented bioactive suppliment (MD 850 Z 2015.08.31) - the heteronuclear coordinative organic compound *sulphate of[Co-III tris-thiosemicarbazidate]•[1,2- (Bi (III) diaminocyclohexane-tetraacetate]] - [Co (thios) 3]•[Bi (cdta)] SO_4 • 66H_2O.*

For bee families of batches III, IV and V, nutritional supplements enriched with coordinative organic compounds containing rare microelements, referred to as CV-6, CV-8 and CV-22, were used.

These symbols are coded by the Chemistry Institute specialists, who synthesized these coordinative organic compounds.

The dry substance of the coordinative organic compounds was diluted in water at a concentration of 1 mg% and mixed with the sugar syrup in a ratio of 2: 100 (20 ml to 1000 ml of syrup) and was administered directly to the bees. To estimate the efficiency of the bee feeding proceeding with the above-mentioned nutritional supplements at the end of May (at first harvest), the morpho-productive characteristics of the bee families in each batch were compared with the control group.

The morpho-productive indices of bee families were determined according to the methodology for assessing the morpho-productive characteristics of the bee families elaborated by us [14] and described in the 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 [19].

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 Plohinskiy N.A. [39].

RESULTS AND DISCUSSIONS

The research results have shown that some of the coordinative organic compounds tested in bee's feed as biologically active substances for enriching nutritional supplements tended to influence some morpho-productive characteristics of reproduction and development of bee families (Tab. 2).

Thus, the **queen prolificacy**, appreciated at the first harvest, increased significantly in the bee families of the II, III and V batches, compared to the control batch. It was found that the queens of families from the batch II (prototype) who received the nutritional supplement enriched with the coordinative organic compound Co + Bi (patented) had a significantly higher prolificacy compared to the control batch, with 138 eggs / 24 hours or 9.9% (td = 2.6; P <0.05).

The highest prolificity (1607±25 eggs/24 hours) was recorded at the bee families of batch III, who received the nutritional supplement enriched with the coordinative organic compound CV-6 (Fig. 1).

These families had a significantly higher prolificacy, compared to those in the control batch, with 219 eggs/24 hours or 15.8% (td = 4.2; P <0.001).

As well, the coordinative organic compound CV-22 also positively influenced the prolificity of the queens.

The queens of bee families of the batch V, which received the nutritional supplement enriched with the CV-22 coordinative organic compound, had a significantly higher prolificity compared to the control group, with 180 eggs/24 hours or 13.0 % (t_d=3,1; P<0,01). Only the coordinative organic compound CV-8, which was used to enrich the nutritional supplement for bee families of group V, had no stimulating effect on the queens prolificity. Compared to the control group, the difference of 47 eggs / 24 hours in this batch, which is 3.4%, does not have statistical significance,

since the criterion of the certainty of differences between two variables of data after Student is below the zero threshold according to the theory error probability predictions (td = 0.9, P> 0.1). These coordination compounds of organic or causing any qualitative and quantitative impact of royal jelly honey secreted by the nurse and distributed in the feed queens.

Table 2 Results of testing nutritional supplements enriched with coordinating compounds

The experimental batch and the symbol of the organic coordinative compound	Nr	The morpho-productive characters				
		At the initial etape M ± m	At first harvest M ± m	The difference of the first harvest, compared to the control		
				d	%	t_d
Queen prolificity, eggs/*24 hours*						
Batch I (control)	10	444.0 ± 14.4	1388 ± 45	-	-	-
Batch II (Co+Bi)	10	455.8 ± 18.1	1526 ± 29	+138	9.9	2.6*
Batch III (CV-6)	10	454.1 ± 13.2	1607 ± 25	+219	15.8	4.2***
Batch IV (CV-8)	10	440.9 ± 14.4	1435 ± 22	+47	3.4	0.9
Batch V (CV-22)	10	443.3 ± 12.4	1568 ± 36	+180	13.0	3.1**
Quantity of capped brood, *hundred cells*						
Batch I (control)	10	53.30 ± 1.73	166.5 ± 5.4	-	-	-
Batch II (Co+Bi)	10	54.70 ± 2.17	183.1 ± 3.4	+16.6	10.0	2.6*
Batch III (CV-6)	10	54.50 ± 1.59	192.8 ± 3.0	+26.3	15.8	4.2***
Batch IV (CV-8)	10	52.90 ± 1.73	172.2 ± 2.7	+5.7	3.4	0.9
Batch V (CV-22)	10	53.20 ± 1.49	188.2 ± 4.3	+21.7	13.0	3.1**
Colony strength, *kg*						
Batch I (control)	10	1.09 ± 0.02	2.40 ± 0.02	-	-	-
Batch II (Co+Bi)	10	1.09 ± 0.03	2.54 ± 0.03	+0.14	5.8	3.9***
Batch III (CV-6)	10	1.09 ± 0.03	2.54 ± 0.03	+0.14	5.8	3.9***
Batch IV (CV-8)	10	1.08 ± 0.03	2.42 ± 0.03	+0.02	0.8	0.5
Batch V (CV-22)	10	1.09 ± 0.03	2.55 ± 0.03	+0.15	6.2	4.2***
Quantity of bee bread, *hundreds of cells*						
Batch I (control)	10	57.50 ± 2.19	97.7 ± 2.1	-	-	-
Batch II (Co+Bi)	10	61.50 ± 1.97	101.6 ± 2.6	+3.9	4.0	1.2
Batch III (CV-6)	10	60.90 ± 1.47	105.4 ± 1.8	+7.7	7.9	2.8**
Batch IV (CV-8)	10	57.20 ± 1.58	99.1 ± 2.1	+1.4	1.4	0.5
Batch V (CV-22)	10	57.40 ± 1.46	100.8 ± 1.5	+3.1	3.2	1.2
Quantity of wax, *number of combs*						
Batch I (control)	10	-	2.69 ± 0.12	-	-	-
Batch II (Co+Bi)	10	-	2.83 ± 0.06	+0.14	5.2	1.1
Batch III (CV-6)	10	-	2.85 ± 0.05	+0.16	5.9	1.2
Batch IV (CV-8)	10	-	2.83 ± 0.06	+0.14	5.2	1.1
Batch V (CV-22)	10	-	2.87 ± 0.06	+0.18	6.7	1.3
Quantity of honey, *kg*						
Batch I (control)	10	4.47 ± 0.12	12.20 ± 0.42	-	-	-
Batch II (Co+Bi)	10	4.62 ± 0.18	13.61 ± 0.29	+1.41	11.6	2.8**
Batch III (CV-6)	10	4.71 ± 0.15	12.73 ± 0.47	+0.53	4.3	0.8
Batch IV (CV-8)	10	4.35 ± 0.13	11.56 ± 0.31	-0.64	-5.3	1.2
Batch V (CV-22)	10	4.37 ± 0.13	12.19 ± 0.36	-0.01	-0.1	0.02

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

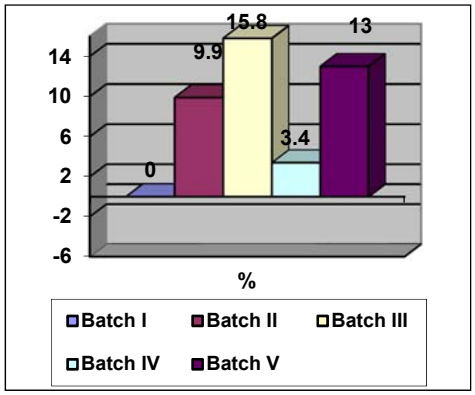

Fig. 1 Diagram of the queens prolificity

The amount of **capped brood**, appreciated in the nest of the families in the experimental batches, at the first harvesting , had a growth rate similar to that of the queens prolificity (Fig.2).

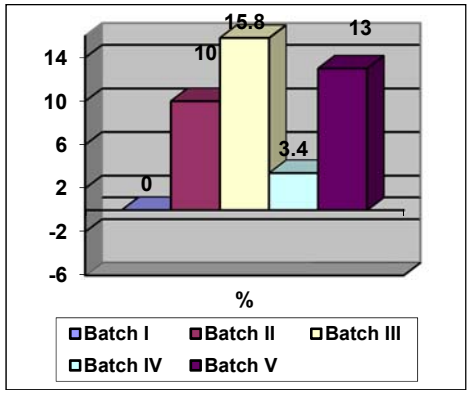

Fig. 2 Diagram of the amount of capped brood

It was found that the coordinative organic compounds used to enrich nutritional supplements for bee feeding in batches II, III and V had a stimulating effect on the amount of capped brood.

Thus, the amount of capped brood from batch II (prototype), which received the nutrition supplement enriched with Co + Bi (patented) organic compound, as we expected, was significantly higher compared to the control batch with 16.6 hundred cells or 10.0% (t_d=2.6; P<0.05). The highest increase of the amount of capped brood was observed in the bee families of batch III. Thus, the amount of capped brood from batch III, which received food supplements enriched with the coordinative organic compound CV-6, was significantly higher compared to the control batch by 26.3 hundred cells or 15.8% (t_d=4.2; P<0.001). A significant increase in the amount of capped brood was also recorded in bee families of group V, which received nutrition supplements enriched with the CV-22 coordinative organic compound. Thus, the amount of capped brood in the families of this batch was significantly higher compared to that of the control batchs families, with 21.7 hundred cells or 13.0% (t_d=3.1; P<0.01). At bee families of batch IV, which received the nutritional supplement enriched with the CV-8 coordinative organic compound, no stimulation effect on the amount of capped brood was established.

The **colony strength** of bees has been influenced by nutritional supplements, depending on the coordinative organic compounds with which they have been enriched. The significant increase in the colony strength, compared to the control group, was established in the same experimental batches, with increased queens prolificity and the amount of capped brood (Fig. 3).

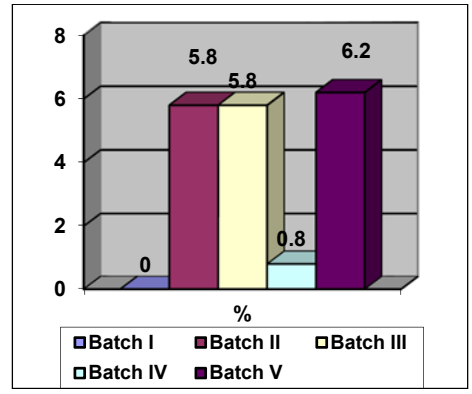

Fig. 3 Diagram of the colony strength

The highest increase in strength was observed in the bee families of batch V, who received the nutritional supplement enriched with the CV-22 coordinative organic compound. Bee families of this batch, at first harvesting, had a higher strength compared to

the control group with 0.15 kg or 6.2% (t_d=4.2; P<0.001). A rather significant increase of strength was also reveled in the bee families of batches II and III, who received in their feed the nutritive supplements, respectively, with Co + Bi and CV-6 coordinative organic compounds. Thus, at the first harvesting, the strength of the bee families in these batches was higher, compared to the control group, by 0.14 kg or 5.8% (td = 3.9, P <0.001).

The **amount of bee bread**, accumulated in the nest at the first harvest, ranged from 99.1±2.1 hundred cells in batch IV, whose families received nutrition supplements enriched with the coordinative organic compound CV-8, up to 105.4 ± 1.8 hundred cells in batch III, whose families received nutrition supplements enriched with the CV-6 coordinative organic compound (Fig. 4).

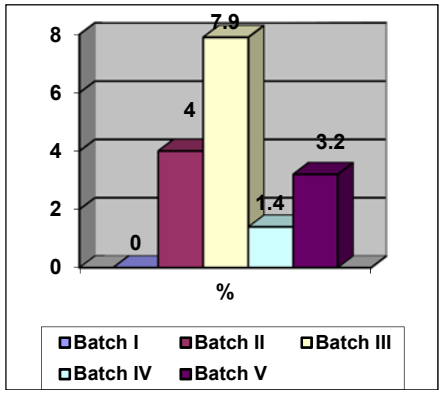

Fig. 4. Diagram of the quantity of bee bread accumulated in the nest

It was found that the amount of bee bread, accumulated in the nest at the first harvest, had changed only at bee families of batch III who received the nutritional supplement enriched with the CV-6 coordinative organic compound.

Thus, the amount of bee bread accumulated in the nest by the bee families of this batch, at the first harvest, significantly exceeded the amount of bee bread gained by the control group, with 7.7 hundreds of cells or 7.9% (t_d=2.8; P<0.01). In the other experimental batches, the amount of bee bread, accumulated in the nest at the first harvest, did not show any significant differences compared to the control batch. So, we can say that the coordinative organic compounds tested by us, with the exception of CV-6, did not stimulate of the bee family's ability to accumulate bee bread in the nest.

The **quantity of bee wax** produced on the first harvesting ranged from 2.69 combs accumulated by bee families in the control batch, which received nutrition supplement only from 50% sugar syrup - unimproved, up to 2.87 honeycombs accumulated in the bee families of group V, who received nutrition supplements enriched with the CV-22 coordinative organic compound (Fig. 5).

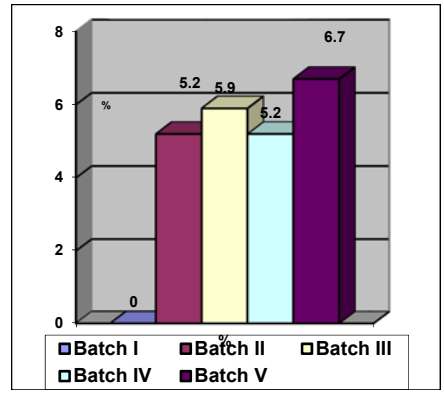

Fig. 5 Diagram of the quantity of wax

We found that significant differences in the amount of wax accumulated in the nest among the bee families in the experimental and control batches were not recorded (t_d=1.1–1.3; P>0.1). Therefore, we can conclude that the coordinative organic compounds tested by us in the bee's food did not somehow influence the secretory functions of the wax-producing mirror glands of worker bees.

The **quantity of honey** accumulated by bee families at the first harvest ranged from 11.56 kg/families in batch IV, which received food supplements enriched with the coordinative organic compound CV-8, up to 13.61 kg/families bees in batch II, who received nutrition supplements enriched with Co + Bi organic coordinative compound (Fig. 6).

It should be mentioned that the coordinative organic compound Co + Bi - patented, confirmed its stimulating property

on the capacity of bee families to accumulate honey production in the nest.

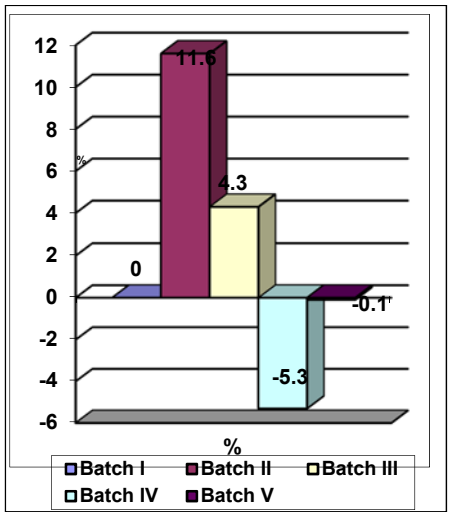

Fig. 6 Diagram of the quantity of honey accumulated in the nest

Thus, as expected, bee families of batch II, who received the nutritional supplement enriched with Co + Bi organic coordinative compound, exceeded their congeners from the control batch after the amount of honey accumulated in the nest at the first harvest, with 1.41 kg or 11.6% (t_d=2.8; P<0.01). There were no significant differences between the bee families in the other experimental batches (III, IV and V) and those in the control batch, according to the amount of honey accumulated in the nest. This means that the coordinative organic compounds tested by us this year did not have any stimulating influence on the capacity of bee families to accumulate the honey in nest.

Generalizing the results of testing the nutritional supplements enriched with coordinative organic compounds containing rare microelements in bees feed, we found that some of these COC (CV-6 and CV-22) had stimulatory properties only on reproductive and developmental characters of bee families. But the stimulating effect of reproductive functions is not certain, because the activation of reproductive and developing characters of bee families must inevitably lead to the increasing of the capacity of accumulation of bee products in the nest. Therefore, none of the newly-tested coordinating compounds showed general stimulatory properties, which would result in increased apiculture production, especially honey production. In this context, the testing of these coordinating organic compounds in bees feed requires repetition.

CONCLUSIONS

1. Two prospective nutritional supplements (CV-6 and CV-22) were previously identified to enrich nutritional suppliments and nourish bees during periods of poor harvesting in nature that had a stimulating influence on the queen's reproductive functions, on increasing of the brood number and the colony strength.

2. At the same time, the results of the increasing reproductive indices of queens and the colony strength did not result in an increase in the amount of apiculture production accumulated in the nest, which makes testing results unreliable in this experiment, requiring a repeated test.

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