Scientific Papers. Series D. Animal Science. Vol. LVIII, 2015
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

INFLUENCE OF SOME ORGANIC COORDINATION COMPOUNDS CONTAINING COBALT AND BISMUTH ON DEVELOPMENT MORPHO-PRODUCTIVE CHARACTERS OF THE BEE FAMILIES

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Abstract

The aim of the research was to evaluate and test the influence of organic coordination compounds containing rare microelements, on the vital functions and on the development of the morpho-character of Apis mellifera bee colonies. Several experiments where conducted and was tested in feeding bees in spring (April 1 to 21) a period with poor harvest in nature, a nutritious blend of 50% sugar syrup supplement enriched with bioactive organic compound heteronuclear coordinative sulfate [tris-thiosemicarbazide cobalt (III)] [1, 2-diaminociclohexantetraacetat bismuth (III)] hexahydrate - [Co (tios) 3], [Bi (CDTA)] $SO_4 \cdot 6H_2O$ in aqueous solution with concentration of 1 mg% (hereinafter referred to as "compound + Co + Bi), which was mixed with sugar syrup in a ratio of 2: 100, and was administered directly into the food of bees and bee families where feed with the nutrient in amount of 100 ... 130 ml for every frame with bees, every 2 days for a period of three weeks. Bee colonies were formed into three lots, each lot formed from 16 families, of which: group I - control, which were fed only with sugar syrup 50%, group II - prototype, which where feed with sugar syrup by adding nutritional supplement enriched with patented "Apispir + Fe + It" (MD 477 Z 2012.09.30) and group III - experimental, which received sugar syrup enriched with supplement "compound + Co + Bi". The research results have shown that using energizing nutritional supplements enriched with biologically active substances of organic compounds coordination in bee feeding in times when there is a poor harvest in nature, helps stimulate vital functions of bee families and increase their productivity: the prolificity of queen - with 5.2 to 9.7% (P <0.001); the amount of capped brood - with 5.2 to 9.7% (P <0.001); family strenght- by 2.5 to 9.7% (P <0.1s P <0.001); disease resistance - by 2.4 to 5.0% (P <0.001); brood viability - with 1.2 to 2.2% (P <0,01s (P <0.001); the amount of accumulated bee bread in brood chamber- by 15.0 to 23.3% (P <0.001), an increased amount of wax combs - by 21.4 to 39.3% (P <0.001) and the amount of honey accumulated in the colony - with 13.9 to 25.4% (P <0.01 and P <0.001). The beneficial effect of feeding bees with biologically active nutritional supplements Apispir + Fe + + Co + Se and Bi compound denotes the fact that in the spring (March-April), a period poor in harvesting, in the area studied, in nature there is a shortage of biologically active substances, including rare microelements.

Key words: *testing, supplements, rare microelements, feeding, bees.*

INTRODUCTION

Many biological research (Istriteanu et al., 2002; Van Strallen, 1994; Войнар, 1960; Игнатьев et al., 2006; Ковальский, 1971; Колосова, 1968; Ноздрюхина, 1977; Тома et al., 1980) performed both in our country and abroad, have proved that that trace elements play an important role in the metabolism of biotic environments both plants and animals. From the numerous number of trace elements, Co is one of the most well studied in the animals and the humans, which can not be said about Bi.

In warm-blooded animal organisms, Co fulfills various functions: synthesis of blood elements, enzymes, vitamin B12 (cyanocobalamin), to boost protein metabolism and assimilation of vitamins A, E, C, activation of some enzymes and antibiotics, inhibition of some pathogenic micro-organisms (Колосова, 1968; Яковлев, 1972). It is assumed that such functions could have cobalt in the insect body through the hem lymph.

The inclusion of cobalt chloride (CoCl2) in bees feed, the researcher Яковлев А.С. 1972 (Яковлев, 1972) obtained an increase of working bees longevity by 5 days compared with controls. Based on this research, the author inferred conclusion that Co strengthens the defence functions of bee's body.

However, a number of other researchers (Hernandes et al., 1985; Somlyay, 1983; Кирилюк, 2006) studied the existence of these trace elements in the biosphere components in terms of heavy metals as environmental pollutants, if their concentration exceeds the maximum allowable. After the particularities of migration in the biosphere, the researcher Кирилюк, 2006 classifies trace Co and Bi in aqueous cations category respectively poorly circulating (low intensity) and less circulating (insufficient), noting their impact, beneficial or harmful on living organisms depends on the concentration and form of their existence in nature and the accessibility of the active circulating forms. For example, bismuth from nature is assimilated by plants very difficult. So, the concentration of the trace element in the flower nectar and pollen is very low. Therefore, we can ascertain, rather, an insufficiency of these rare micronutrients in the nature, but extremely important for living organisms, including bees, than heavy metal pollution. In the spring, after wintering colony of bees, the bee's body there is a deficiency of bioactive nutrients, especially micronutrients, which have a catalytic role in physiological processes of vital activity of bees, fulfilling multiple functions in the bees body at the cellular level, entering into the composition of enzymes and hormones with decisive role in metabolism. Insufficient of active biological substances, especially trace elements, leading to the weakening of resistance to diseases of bee families and decrease their productivity (Колосова, 1968).

The main natural sources of supply the body with micronutrients are bees nectar and pollen collected from melliferious plants. According to scientific information in honey and pollen are more than 30 micro-macro, including trace elements. Among them, cobalt and bismuth are found in very small quantities, from 10 to 14 mg%, but their role in the metabolism of substances in living organisms is enormous.

Given that the spring (poor harvesting period in kind), most beekeepers feeds bee families with sugar syrup, which constituted more than half of the necessary trace elements are missing, then identify sources of available trace elements to enrich ration nutritional supplement of bees families becomes an actual problem.

Are known processes for the feeding of bee families sources with trace elements, in particular in salt form of cobalt $CoCl_2$ at a dose of 8-25 mg per litre of sugar syrup (Яковлев, 1972), but the efficiency of these methods is very low, because digestion and assimilation of this salt in the body of bioactive substances are very weak.

For these reasons, researchers of the Institute of Zoology of the ASM, together with those from Moldova State University, undertook a series of studies focused on the identification of available sources of biologically active substances to feed bees, including trace elements, obtained using coordination of organic compounds (Patent MD 475, 476, 477 Z 2012; Cebotari et al., 2012; Cebotari et al., 2013; Cebotari et al., 2013) According to the information of Заозерский (1965), coordination complexes compounds plays an enormous role in the processes of vital activity of living organisms. Such substances extremely important as regards biological, such as haemoglobin and chlorophyll are into complex compounds. It was observed that a number of rare elements which are found in animal and plant tissues, enter into the coordination complex compounds. Metals linked in complex are part of the enzymes, in particular to those oxidative.

In this context, the aim of the research was to assess the influence of some coordination organic compounds containing rare trace elements, on the vital functions and development morph productive character of *Apis mellifera* bee families.

MATERIALS AND METHODS

The researches were conducted on families of bees *Apis mellifera carpathian* grown at experimental apiary of Zoological Institute of the Academy of Sciences. Apiary is located at stationary in a clearing of the forest, near its edge. The main melliferious sources in this area

are white acacia, linden and spontaneous flora inclusive yellow melilot.

In special, experiments was tested in bees feeding in spring (April 1 to 21) from poor harvesting in nature, a nutrient mixture of 50% sugar syrup supplement enriched with bioactive supplement from hetero nuclear organic coordination compounds *sulphate [tris-thiosemicarbazide of cobalt (III)] [1, 2-diaminociclohexantetraacetat of bismuth (III)] hex hydrate - [Co (tios) 3], [Bi (CDTA)]* $SO_4 \cdot 6H_2O$ in aqueous solution to the concentration of 1 mg% (hereinafter referred to as "Compound + Co + Bi), which was mixed with sugar syrup in a ratio of 2: 100, administered directly into bees food, and bee families feeding with nutrient mixture was carried out at 100 ... 130 ml of mixture at every frames interval populated with bees, every 2 days for three weeks.

To estimate the efficiency of the process of bees feeding with up-nominated nutritional supplement, experiments were conducted comparative testing it on bee colonies formed in three batches, each batch by 16 families in each group, which batch I - control, which: bees were fed only 50% sugar syrup only, batch II - prototype, the bees which received in food sugar syrup enriched with nutritional supplement patented "Apispir + Fe + Se" (MD 477 Z 2012.09.30) obtaining from *Spirulina platensis* biomass grown in the presence of organic coordination compounds *selenite Fe (III) chloride hex hydrate* $FeSeO_3 \cdot 6H_2O$ and batch III - the actual experiment, the bees which received the sugar syrup enriched with food supplement *"Compound + Co + Bi"*.

After spring stimulation to the 45 days of the experiment start and first collected (70 days after the start of the experiment), at the bee families were evaluated the following morpho - productive characters: queen´s prolificacy, brood´s capacity, family strength, resistance to disease, brood´s viability, increased amount of wax combs in the nest, the quantity of honey and bee bread accumulated in the nest.

Determining the level of development bee families morpho-productive character were carried, out according to the methodology developed by us (Cebotari, 2010) for livestock norm concerning bee families assessment, growth and certification of beekeeping genitor material certification, approved by Government Decision no. 306 of 28.04.2011 (OJ no. 78-81 of 13.05.2011, art. 366) (Livestock norm, 2011). The data obtained in experience, was statistically processed using computer software "STATISTICA - 6" and was evaluated their certainty, according to variation biometric statistics, by the methods of Плохинский (1969).

RESULTS AND DISCUSSIONS

The test results in bees feeding, of nutritional supplements enriched with aqueous solutions of coordination organic compounds containing rare trace elements, demonstrated that they (supplements) had a beneficial action in general, on vital activity of bee colonies and growing, especially, their productivity (Table 1).

Mentioned that in the 45 days after the beginning of the experiment, the bee families of experimental groups who received in feed nutritional supplements enriched with coordination organic compounds containing rare microelements with both „*Apispir + Fe + Se*", and the *"Compound + Co + Bi",* had only a rising trend compared with controls batch the level of character and morpho- productive feature, within the limits 1.7 - 4.0% (P>0.1). Given the fact that the coefficient of certainty of these differences was recorded as zero the threshold level of probability theory of forecasts contest without error after Student (Плохинский, 1969) meaning beneficial effect at this stage (45 days after the start of the experiment), gives only a tendency character. At the same time, the period of 70 days from the start of the experiment (see Table first collected), beneficial influence of biologically active substances of organic coordination compounds supplement *"Apispir + Fe + Se",* as well the appendix "*Compound + Co + Bi"* has become significant, and the certainty coefficient of difference between of bee families value morpho-productive characters from experimental batches and control batch when reached the highest certainty threshold according to the probability theory of forecasts contest without error.

Table 1. The test results in bees feeding (first harvest) with nutrient supplements, enriched with active organic coordination compounds, containing rare microelements.

The experimental batch and bioactive element	The number of families in batch, N	The average value of character **M ± m**	The difference from the control batch		The coefficient of certainty, t_d
			d	%	
Queens prolificacy, eggs/24 hour					
Batch I (control)	16	1590 ± 20	-	-	-
Batch II (*Apispir+Fe+Se*)	16	1673 ± 14	+ 83	5.2	3.4**
Batch III (*Compound+Co+Bi*)	16	1745 ± 14	+ 155	9.7	6.3***
Quantity of capped brood, hundred cells					
Batch I (control)	16	190.8 ± 2.4	-	-	-
Batch II (*Apispir+Fe+Se*)	16	200.7 ± 1.6	+ 9.9	5.2	3.4**
Batch III (*Compound+Co+Bi*)	16	209.4 ± 1.6	+ 18.6	9.7	6.4***
Family strength, kg					
Batch I (control)	16	3.20 ± 0.02	-	-	-
Batch II (*Apispir+Fe+Se*)	16	3.28 ± 0.04	+ 0.08	2.5	1.7
Batch III (*Compound+Co+Bi*)	16	3.51 ± 0.05	+ 0.31	9.7	5.8***
Resistance to disease, %					
Batch I (control)	16	88,4 ± 0,4	-	-	-
Batch II (*Apispir+Fe+Se*)	16	90,5 ± 0,3	+ 2,1	2,4	4,2***
Batch III (*Compound+Co+Bi*)	16	92,8 ± 0,3	+ 4,4	5,0	8,8***
Broods viability, %					
Batch I (control)	16	89.3 ± 0.3	-	-	-
Batch II (*Apispir+Fe+Se*)	16	90.4 ± 0.3	+1.1	1.2	2.6**
Batch III (*Compound+Co+Bi*)	16	91.3 ± 0.4	+2.0	2.2	4.0***
Quantity of bread, hundreds of cells					
Batch I (control)	16	90.5 ± 1.8	-	-	-
Batch II (*Apispir+Fe+Se*)	16	104.1 ± 1.5	+ 13.6	15.0	5.8***
Batch III (*Compound+Co+Bi*)	16	111.6 ± 2.1	+ 21.1	23.3	7.6***
Quantity of wax, kg					
Batch I (control)	16	0.28 ± 0.01	-	-	-
Batch II (*Apispir+Fe+Se*)	16	0.34 ± 0.01	+ 0.06	21.4	4.3***
Batch III (*Compound+Co+Bi*)	16	0.39 ± 0.01	+ 0.11	39.3	7.8***
Quantity of honey, kg					
Batch I (control)	16	11.62 ± 0.40	-	-	-
Batch II (*Apispir+Fe+Se*)	16	13.24 ± 0.40	+ 1.62	13.9	2.9**
Batch III (*Compound+Co+Bi*)	16	14.57 ± 0.38	+ 2.95	25.4	5.3***

Notice: * P < 0.05; ** P < 0.01; *** P < 0.001.

So, the **queens prolificacy** from bee families, being identical in all batches at the beginning of the experience, then increased significantly at the first harvest, compared with control batch, in the experimental batches, whose families have received the feed supplement enriched with both *Apispir + Fe + Se*, and *Compound + Co + Bi.* the coordinating organic compound. The most pronounced increase queens prolificacy was recorded in batch III, bees were fed during spring (April) with energy nutritional supplements, enriched with organic coordination compounds which contained rare microelements *Compound+Co+Bi*. The Queens of bee families from this batch exceeded certainly after prolificacy, their congeners from the control group - with 155 eggs or 9.7% (td=6.3; P<0.001). Be mentioned, that the difference, compared with the control batch, the queen prolificacy from bee families in the batch III, who received in feed nutritional supplement enriched with the coordinating organic compound containing rare microelements (Co, Bi) was 1.9 times higher, than the queen bee families in batch II, who received feed supplement enriched with *Apispir + Fe + Se* (P<0.001).

The quantity of capped brood, as determined by the queen prolificacy, as also positively influenced by nutritional supplements enriched with both biomass extract *Apispir + Fe + Se,* as well the coordinating organic compound containing rare trace elements such as *Compound + Co + Bi.* Thus, the bee families from experimental batches significantly exceeded after this character in the first harvest their congener from the control batch. The highest increase in the amount of capped brood,

compared with controls, was registered at the bee families from the batch III, who received in feed nutritional supplement enriched with the coordinating organic compound containing rare microelements *Compound + Co + Bi*. The difference in increasing amount of brood capped in the bee families from this batch compared with the control batch was 18.6 hundred cells, or 9.7%. This difference is certain, with the highest certainty threshold of the probability theory of forecasts without error after Student (td= 6.4; P<0.001).

Thus, the strange of bee families, being identical in all groups at the beginning of the experience, then, at the first harvest, significantly increased compared with controls, in experimental lots, whose families received in feed nutritional supplements enriched, both the biomass extract *Apispir + Fe + Se,* and with the coordinating organic compound containing rare microelements (Co, Bi). The most pronounced increase of the strange of bee families was recorded in group III; the bees were fed during spring (April) with energy nutritional supplement enriched with coordination compounds *Compound + Co + Bi*. The Bee families from this lot exceeded with certainty, after power, their congeners from the control group - with 0.31 kg or 9.7% (td=5.8; P<0.001). Be mentioned, that the difference compared with the control group, the power from group III bee families, who received in feed nutritional supplement enriched with the coordinating organic compound containing rare microelements (Co, Bi), was about 3.9 times higher, than in bee families from batch II, who received feed supplement enriched with *Apispir + Fe + Se.*

Resistance to diseases of bee families, as a biological trait determined hereditary, can be influenced, at the same time, by external factors, among which, the most important is nutrition. The experiment results showed that at the first harvest, the bee families from experimental batches had a higher resistance, compared with controls batch. As a high resistance to disease was recorded in the bee families from the batch III, who received in feed nutritional supplement enriched with organic coordination compounds which contained rare microelements *Compound + Co + Bi*. After the value of this character, the bee families from this experimental group exceeded their congener from the control batch with 4.4 percentage points or 5.0% (td= 8.8; P<0.001). Given the fact, that biological variability of this character is very narrow, significance of this difference (small at first glance, the absolute size) is quite high and corresponds to a high certainty threshold of the probability theory forecasts contest without error.

The more obvious, this increased of development morpho-productive characters of bee families from experimental batches can be seen in the histogram (see figure). From the diagram it can be seen that all the morpho-productive characters of the bee families from experimental lots, shown in brown and yellow colours pillars is significantly higher than the control batch.

The broods viability, such as disease resistance, the bee families from bees whose experimental lots received feed supplements enriched with biologically active substances, was significantly higher compared with batch controls. The highest viability of brood was recorded in the bee families from the batch III, who received in feed nutritional supplement enriched with organic coordination compounds, *Compound + Co + Bi.* After this important biological character, the bee families from the batch III significantly exceeded their contemporaries from the control group with 2.0 or 2.2%. Because brood viability character has a narrow variability (as disease resistance), this small difference at first sight is certainly the highest threshold (td=4.0; P<0.001), according to forecasts contest without error probability theory.

The quantity of bee bread accumulated in nest was also positively influenced by nutritional supplements enriched, both biomass extract *Apispir + Fe + Se*, and with the coordinative organic coordination compounds rare microelements such as Co and Bi. Thus, the bee families from experimental lots significantly exceeded by the level of this character, at the first harvest, their congener from the control group with 13.6-21.1 hundred cells, or 15.0-23.3% (P <0.001). The highest increase amount of bee bread accumulated in the nest, compared with controls, was registered that the bee families from the batch III, who received in feed nutritional supplement enriched with the

coordinative organic compound containing rare microelements *Compound + Co + Bi.* The difference in increasing amount of brood capped in the bee families from this batch, compared to with the control batch is 21.1 hundred cells or 23.3%. This difference is certain with the highest certainty threshold of probability forecasts without error (td=7.6; P<0.001).

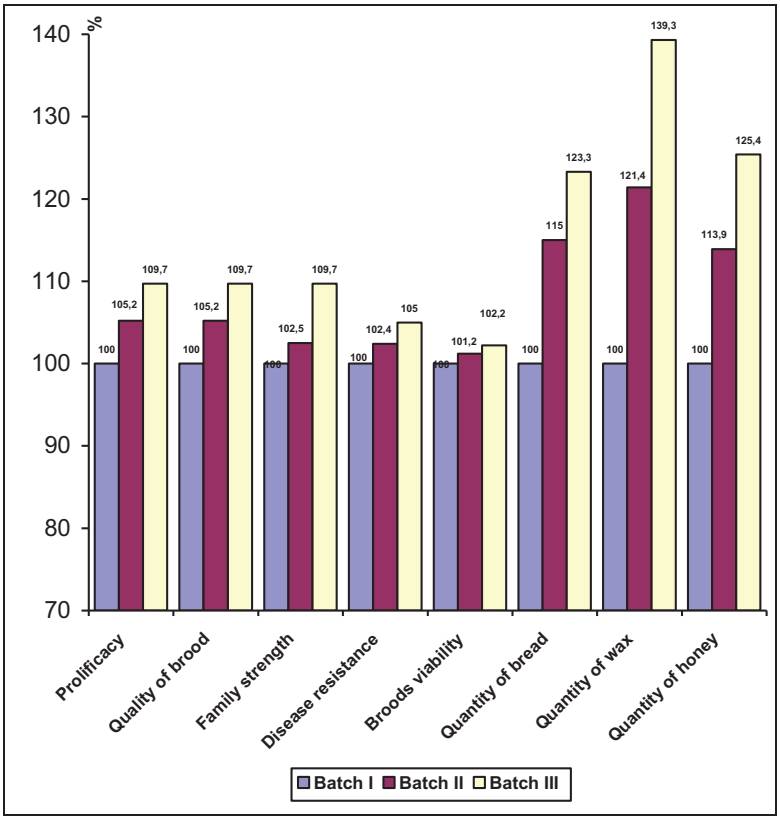

Figure 1. The level of morpho- productive characters of the bee families, compared with control batch.

The quantity of wax increased on the combs in the nest was equally influenced positively by nutritional supplements enriched with both biomass extract *Apispir + Fe + Se,* and with the organic coordination compounds containing microelements such as Co and Bi.

Thus, the bee families from experimental lots significantly exceeded by the level of this character, at the first harvest, their congener from the control batch with 0.06-0.11 kg or 21.4-39.3% (P<0.001). The highest increase of wax quantity in the nest, compared with controls, was registered to the bee families from the batch III, who received in feed nutritional supplement enriched with the organic coordination compounds containing rare microelements (Co + Bi). The difference in increase, compared to controls, of wax quantity accumulated in the nest bee families from this lot, was 0.11 kg, or 39.3%. This difference is certain with the highest certainty threshold of the probability forecasts without error (td=7.8; P<0.001).

The quantity of honey accumulated in the nest, is the most important morpho-productive character, was also positively influenced by the nutritional supplements, enriched with the extract of biomass as *Apispir + Fe + Se,* and with the organic coordination compounds containing rare microelements such as Co and Bi.

Thus, the bee families from experimental lots II and III significantly exceeded level of production of this character at the first harvest, their congener from the control group with 13.9-25.4% (P<0.01 and P<0.001). Of these two

groups, the largest increase in the amount of honey accumulated in the nest, compared with controls, was registered at the bee families from batch III, who received in feed nutritional supplement enriched with organic coordination compounds containing rare microelements *Compound + Co + Bi*. The difference in growth of the amount of honey accumulated in the nest bee families from this batch, compared with controls, is 2.95 kg or 25.4%. This difference is certain, with the highest certainty threshold of the probability theory of forecasts without error after Stiudent (td=5, 3; P< 0,001).

Generalizing the results of research development level of morpho-productive character of bee families can conclude, that biologically active substances from organic coordination compounds mentioned above, certainly contributes to the activation of family reproductive functions (queens prolificacy, quantity of capped brood, family strength), strengthening immunity of body insect (increasing resistance to disease and brood viability) and substantially increasing as a whole productivity of bee families. Beneficial influence of coordinative organic compounds on the vital functions of bee families is explained by us, not only by the action of rare microelements, and through the complex action of all biologically active substances from their complicated molecular structure with their structural ties very close and stable, including, of the complexes ions of the ligands and metal ions of the radicals with modified valence and increased penetration properties of cell membranes of living tissues of the bee body.

CONCLUSIONS

Using bees in feed during periods of poor harvest in nature, of energizing nutritional supplements, enriched with biologically active organic compounds of organic coordination compounds hetero nuclear sulphate [tris-thiosemicarbazide of cobalt (III)] [1, 2 – diaminociclohexan tetraacetat of bismuth (III)] hex hydrate - [Co (tios) 3] [Bi (CDTA)] $SO_4 \cdot 6H_2O$, contributes to stimulating the vital functions of bee families and increase to their productivity: the queen prolificacy - 9.7%; the amount of capped brood - 9.7%; family strength - with 9.7%; resistance to disease - 5.0%; the brood viability - with 2.2%; the quantity of bees bread accumulated in the nest – with 23.3%; increased amount of wax combs - by 39.3% the honey quantity honey gathered in the nest - by 25.4%.

The beneficial effect of the feeding bees of biologically active nutritional supplements Apispir + Fe + Co + Se and Bi compound indicates, that in the spring (March-April) poor harvesting in the studied area, in nature there is a shortage of biologically active substances, including rare microelements.

The deficit of biologically active substances in bees ration can be completed by synthesizing new organic coordinative compounds by new generation, highly effective in stimulating vital activity processes of *Apis mellifera* bee families.

ACKNOWLEDGEMENTS

The work was performed under the project of basic scientific research 14.02.219F "Diversity, structure and functioning of natural and anthropogenic faunal complexes in the context of strengthening national security and strategy of the Republic of Moldova".

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