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Feeding of the Bee Families with an Addition of $CoSO_4$

Research Article

R. Balkanska[1*] and D. Salkova[2]

[1] Institute of Animal Science, Spirka pochivka 1, 2232 Kostinbrod, Bulgaria
[2] Deparment of Experimental Parasitology, Institute of Experimental Morphology, Pathology and Antropology with Museum, Bulgarian Academy of Science, Sofia, Bulgaria

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*Correspondence E-mail: r.balkanska@gmail.com

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ABSTRACT

For their normal living, honey bees (*Apis mellifera*) require proteins, carbohydrates, lipids, vitamins and minerals. It is well known that Co and vitamin B_{12} are two nutrients that have been reported in pollen and plants that have had positive results in bees feeding. In this respect, the aim of the study is to determine the influence of feeding of the bee families with $CoSO_4$ as a supplement on the chemical composition of non-flying worker bees' bodies and some bee products components. The study was conducted during May-August 2015 in the experimental apiary of the Institute of Animal Science, Kostinbrod. A total of 6 bee families were observed-3 experimental (fed with sugar syrup, sugar: water 1:1 and 4 mg/L $CoSO_4$ as a supplement) and 3 control (fed only with sugar syrup). The results show that the addition of 4 mg/L $CoSO_4$ in the sugar syrup of the bee families do not have influence on the average weight and chemical composition of non-flying worker bees. Statistically significant differences ($P<0.05$) in the diastase activity of the honey in the experimental group bee families were found.

KEY WORDS bee haemolymph, bee products, $CoSO_4$, lysozyme, total protein.

INTRODUCTION

For their normal living, honey bees (*Apis mellifera*) require proteins, carbohydrates, lipids, vitamins and minerals. They receive all of these components from the nectar and the bee pollen. At the same time, in order to increase the average productivity, activation of the bee queen laying activity, more brood breeding and development of the hypopharyngeal glands, it is increasingly necessary various supplements in the beekeeping to be used. According to their chemical nature these supplements are proteins and amino acids (Nenchev and Zhelyazkova, 2010), carbohydrates and their substitutes (Ivanova, 2005), vitamins (Zhelyazkova and Nenchev, 1995; Zhelyazkova and Nenchev, 2001), trace elements (Simkus *et al.* 2007), various stimulating products (Shumkova, 2016), plant extracts (Hristakov, 2012) and a combination of vitamins and minerals (Stoilov *et al.* 2000; Sahinler *et al.* 2005). However, the effect of different application of trace elements such as Co, Mn, I and Se to the bees' life processes are studied mainly for increasing the bee brood. Furthermore, the trace elements in the beekeeping practice are considered to be stimulators of the resistance and the development of the immune system. The importance of Co salts was underlined for the first time by a few authors from the field of bee nutrition (Glushkov and Yakovlev, 1965; Goloskov, 1977). It is well known that Co and vitamin B_{12} are two nutrients that have been reported in pollen and plants that have had positive results in bees feeding. The presence of Co in the diet increased the number of bee larvae in bee families with 29.24% (Colibar *et al.* 2011). Some veterinary medical products (Startovit) which contain Co salts also increase the strength of the bee colonies and quantity of the bee brood (Zhelyazkova *et al.* 2008). In Bulgaria Zhelyazkova (1999) and Zhelyazkova

(2005) found that the addition of Co (6 mg/L) and Mn (2 mg/L) salts in the bee food had a positive impact on the development and productivity of the bee families and vitamin C metabolism in the bee organism.

The review of the available literature shows that there is no comprehensive and systematic study for the effect of $CoSO_4$ as a supplement on the chemical composition of the bee bodies and the bee products obtained. In this regard, the aim of the study is to determine the influence of feeding of the bee families with addition of $CoSO_4$ on the chemical composition of non-flying worker bees' bodies and some bee products components.

MATERIALS AND METHODS

The study was conducted during May-August 2015 in the experimental apiary of the Institute of Animal Science, Kostinbrod. Before the experiment the bee families were balanced in regards to strength. In the experiment for royal jelly production a total of 6 bee families were observed-3 experimental (fed with sugar syrup, sugar: water 1:1 and 4 mg/L $CoSO_4$ as a supplement) and 3 control (fed only with sugar syrup). The royal jelly is obtained by the artificial wax cups method (Grout, 1992). The sugar syrup is administered in a dose of 300 mL, 3 times per week in the bee feeders of the experimental and control group bee families. The following parameters were measured during the experiment: chemical composition of non-flying worker bee bodies – water content and mineral composition-weight analysis; proteins-Keldahl method, lipids-Soxhlet method (AOAC, 2002). The bee samples were collected at the end of the experimental period. The bees are anesthetized with diethyl ether and stored in a refrigerator at -20 ˚C. Average body weight of non-flying worker bees (g) - 100 honey bees were collected from each bee family from different honeycombs. The weight of each bee is measured on an analytical balance.

Total protein in the bee haemolymph - spectrophotometric determination by the Audit Diagnostics Kit and the lysozyme content was determined by the method of Motavkina *et al.* (1979). The sample analysis was carried out at the National Diagnostic Science and Research Veterinary Medical Institute, Sofia.

Average number of queen cells – the smallest number of queen cells in each bee family is determined. From all bee families royal jelly is collected from the same number of queen cells (the smallest number of queen cells). The average queen cells number is obtained as the arithmetic average of all processes for royal jelly production.

Average amount of royal jelly in a queen cell - it represents the total amount of royal jelly divided by the queen cell numbers. The average quantity is obtained as the arithmetic average of all samples obtained.

Physicochemical properties of multifloral honey – water content, electrical conductivity, diastase activity and hydroxymethylfurfural (HMF) are determined according to the Harmonised methods of the European Honey Commission (Bogdanov *et al.* 1997).

Content of Co – royal jelly and multifloral honey samples were digested with concentrated HNO_3. The Co content was analyzed with a flame atomic absorption spectrometer Perkin Elmer Analyst 400 with an air / acetylene flame.

The optimal instrumental parameters are determined according to the manufacturer's instructions. The analytical signal of Co is periodically checked with a known concentration standard.

The statistical analysis was done using SPSS, version 21 for windows (SPSS, 2012). The results are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

In Table 1 are presented the results for chemical composition of non-flying worker bees. The analysis of the data shows that the protein, lipid and mineral content are not significant between control and experimental groups after feeding of the bee families with $CoSO_4$ as a supplement. The amount of protein of the bee bodies increased on average by 3%. Similar results are obtained by Simkus *et al.* (2007) for the effect of organic selenium preparation Selenopiran on the development and productivity of bee families. The amount of protein of the bee bodies increased on average by 3%.

No significant difference was found in the average weight of non-flying worker bees fed sugar syrup with $CoSO_4$. The mean weight and standard deviation in the control group was 0.108 ± 0.018 g and in the experimental group was 0.112 ± 0.010 g. Figure 1 shows the minimum and maximum values of the tested indicator for the two groups. The results show narrower ranges of variation in the mean weights of worker bees in the experimental group compared to the control.

To establish the effect of $CoSO_4$ in the sugar syrup, the total protein and lysozyme in the haemolymph of non-flying worker bees were examined. The obtained haemolymph samples are assembly samples of a large number of bees. The results for the amount of total protein and lysozyme in bee haemolymph are presented on Figures 2 and 3.

The results for the total protein and lysozyme content between the experimental and the control group are identical. In this experiment the addition of $CoSO_4$ do not influence the synthesis and accumulation of total protein and lysozyme content in the bee haemolymph.

Table 1 Chemical composition of the body of non-flying worker bees (Mean±SD)

Parameter (%)	Control group (n=350)	$CoSO_4$-supplemented group (n=350)
Water content	67.27±0.61	68.27±3.23
Dry matter	32.73±0.61	31.74±3.23
Protein	53.63±0.66	56.08±3.39
Lipids	4.25±0.25	4.78±0.84
Ash	4.10±0.71	4.68±0.10

SD: standard deviation.

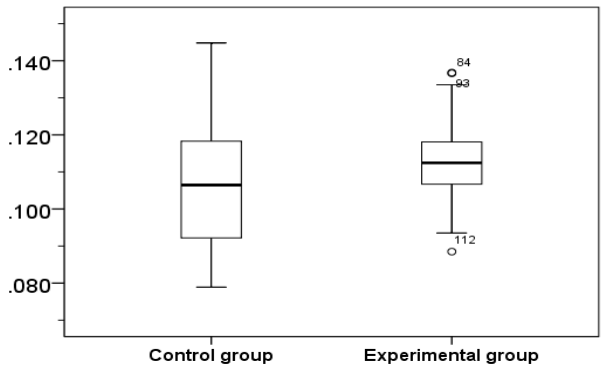

Figure 1 Box plot diagram of the average weight of non-flying worker bees, g, (n=100)
Mean values ± standard deviations in the control group – 0.108 ± 0.018 g; experimental group – 0.112 ± 0.010 g, not significant differences

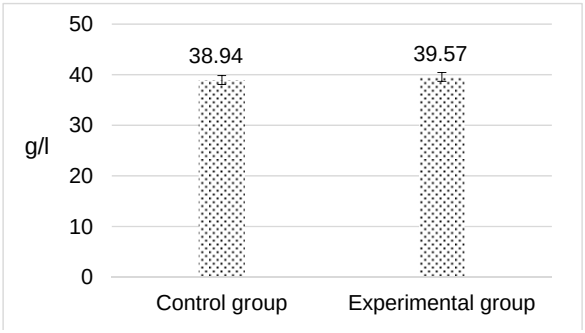

Figure 2 Total protein content in bee haemolymph, (n=150), not significant differences

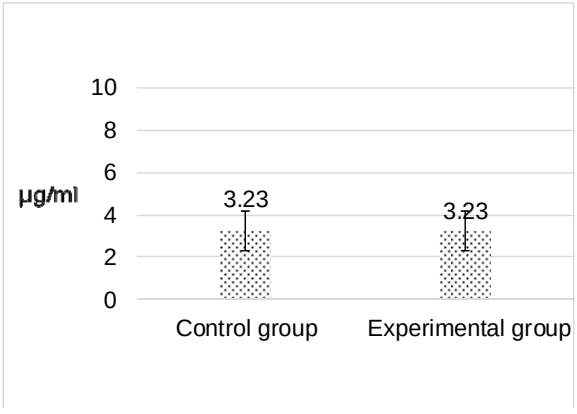

Figure 3 Lysozyme content in bee haemolymph, (n=150), not significant differences

It is well known that the total protein and lysozyme values in the haemolymph can be used to detect the immunostimulatory effect of various biologically active substances (Gurgulova *et al.* 2001). Until now, in the available literature, there is no data on the content of these two haemolymph components in the bee families fed with $CoSO_4$. Table 2 shows the results for the average quantity of royal jelly in a queen cell.

Table 2 Number of queen cells and quantity of royal jelly (Mean±SD)

Parameter	Control group (n=7)	$CoSO_4$-supplemented group (n=7)
Average number of queen cells	82	82
Average quantity of royal jelly in a queen cell, g	0.140±0.022	0.139±0.038

SD: standard deviation.

No significant differences in the average amount of royal jelly produced in the control and the experimental group were found. This means that the $CoSO_4$ as a supplement in the sugar syrup does not lead to a larger quantity of royal jelly compared to the control group. The next items are connected to some components of the composition of the bee products obtained. It is interesting to note that $CoSO_4$ added to the sugar syrup influences some physicochemical characteristics of bee honey. From all studied parameters in the honey samples - water content, electrical conductivity, hydroxymethylfurfural, only diastase activity showed statistically higher values in the samples obtained from the experimental group ($P<0.05$), (Table 3).

Table 3 Physicochemical properties of multifloral bee honey (Mean±SD)

Parameter	Control group (n=5)	$CoSO_4$-supplemented group (n=5)
Water content, %	16.62±1.00	15.80±0.97
Electrical conductivity, µS/cm	415.60±22.52	439.80±24.53
Diastase activity, Gothe units	23.15±3.57^b	27.58±1.66^a
Hydroxymethylfurfural, mg/kg	3.80±0.91	2.96±0.79

The means within the same row with at least one common letter, do not have significant difference ($P>0.05$).
SD: standard deviation.

Processing of nectar into honey and in royal jelly synthesis involve the same glands, which perform various functions during the different stages of the worker bees' development.

From the results obtained, it can be assumed that the bee families fed with $CoSO_4$ influence on the hypopharyngeal glands of worker bees. As a result they are better developed and the honey from the experimental bee families has a higher diastase activity than the control group. Simkus *et al.* (2007) found that diastase activity in the bee honey from the experimental group bee families (fed with Selenopiran as a supplement) is higher than in the control group but the results are not significant.

The addition of $CoSO_4$ on the experimental group did not affect significantly the Co content of the studied bee products (Table 4).

Table 4 Content of Co in bee products (Mean±SD)

Bee product	Control group (µg/g)	$CoSO_4$-supplemented group (µg/g)
Royal jelly, (n=6)	0.09±0.05	0.12±0.09
Multifloral bee honey, (n=5)	0.55±0.06	0.50±0.08

SD: standard deviation.

The differences in the Co content of royal jelly and honey from the experimental and control groups are not statistically significant. Zhelyazkova *et al.* (2010) also found lower values for Co content in honey samples. As a major reason for the non-accumulation of Co in royal jelly and honey can be explain with the bio-barrier function of the bee organism.

CONCLUSION

The addition of 4 mg/L $CoSO_4$ in sugar syrup do not effect on the average weight of non-flying worker bees and the chemical composition of their body. Statistically significant differences ($P<0.05$) in the diastase activity of the honey in the experimental group bee families were found. The addition of $CoSO_4$ on the experimental group did not affect significantly the Co content in royal jelly and multifloral bee honey.

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