

Content of mineral elements in honey and its biological value in conditions of feeding to bees of citrates of Co, Ni, Ag and Cu

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The purpose. To determine influence of citrates of Co, Ni, Ag and Cu on the content of separate mineral elements in honey and to assess its quality. **Methods.** In samples of honey they determined content of Cu, Fe, Co, Cr, Zn, Pb, Cd using atomic-absorption spectrophotometer of SF-115 PK, and physicochemical parameters of honey using conventional procedures. **Results.** Application of citrates of microelements with sugar syrup for supplementary feeding of honeybees testified to correcting impact of citrates on the content of separate beneficial and toxic elements in honey and its biological value during ripening. At the same time there were changes of quality content of honey of the probed groups. **Conclusions.** Positive changes in samples of both probed groups were gained in the content of Fe, Cu, Cr, Pb and Cd in honey, as well as its physicochemical parameters and quality with heightening the content of proline, diastase number and pH content. It confirmed expediency of use of addition of citrates of Co, Ni, Ag and Cu for the purpose of correction of processes of mineral nutrition of honeybees and quality of their produce that is more expressed at addition of Ag and Cu to sugar syrup.

Key words: *honeybees, inorganic substances, citrates, proline, diastase number, weight fraction of water, pH.*

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Improving the quality of food products is an objective process, caused by a large number of regulations and high level of control and with ever-increasing demands of the consumers. The quality of honey is a concept that covers a fairly broad range of characteristics and properties of this unique product. Natural biochemical processes that improve its biological value and quality take place during the storage of honey from the moment of pumping. As a result, its content is constantly changing, and the product itself is called "alive" [1-3]. Like any food product, bee honey is characterized by organoleptic and physico-chemical indicators that determine its quality. Moreover, indicators of honey safety are defined - toxicity, heavy metals, pesticides, antibiotics and radionuclides content [4]. Organoleptic quality indices are specified in DSTU 4497: 2005 "Natural honey. Technical Requirements". Under the condition of their control color, taste, aroma, consistency, crystallization, signs of fermentation of the product and mechanical impurities are determined [5, 6].

Currently, the search continues for new drugs and biologically active additives used in feeding of melliferous bees in order to increase their resistance and improve the biological value and quality of products. In recent decades, a priority direction in nanotechnology was created in Ukraine. It helped to obtain carboxylates of the main nutritional acids of biogenic metals such as Zn, Mg, Fe, Cu, Co, Cr, Se, CO, Ni and Ag [7, 8]. Studies in veterinary medicine established that nanocarboxylates of these trace elements are not toxic. Ions of metals from such complexes are quickly and effectively assimilated by the living organism as vitally necessary trace elements. The expediency of their application is proved not only for obtaining biocidal effect, but also as active nanotechnological compounds that are much more effective than trace elements in classical ionized form in the field of beekeeping [9,10]. Taking this into consideration, studies concerning the increase of biological value and quality of beekeeping products, including honey using trace elements citrates in feeding bees are topical.

The purpose of the study is to compare the influence of Co, Ni, Ag and Cu citrates on the content of separate mineral elements in honey and evaluation of its quality.

Materials and methods of the research. The research was conducted in two stages. First stage - the experiment was carried out in the conditions of stationary beekeeping on the basis of apiaries of the Institute of Animal Biology of the NAAS of Ukraine. The influence of Co and Ni citrates at their introduction into sugar feeding in the summer-autumn (August-September) period was investigated. To carry out the research on the apiary three bee families were selected - three bee families - hives in each. I control group - the conditions of stationary keeping with additional feeding during 14 days 0,3 l / week / family of sugar syrup, group II - under similar conditions to I group of sugar syrup feeding with addition of citrate Co in a dose of 2 mg, group III - under similar conditions of sugar syrup feeding (0.3 l / week / family) with addition of Ni citrate in a dose of 1 mg.

Stage II - conducted on the basis of the Lviv National University of Veterinary Medicine and Biotechnology named after S. Z. Gzhytsky apiaries, which were held in multi-hull 8-frame hives. The influence of Ag and Cu citrates on their introduction into sugar feeding in the spring-summer (April-May) period was investigated. Three bee colonies in each group were selected for the research on the apiary. Control group (I) bees were fed with sugar syrup (1000 ml /week/bee family), II experimental group was kept under similar conditions to I group and fed with sugar syrup with the addition of 0.2 mg of Ag and 0.2 mg of Cu as citrate, the III experimental group - under similar conditions received Ag and Cu in the form of citrate, in doses of 0.5 mg each per 1000 ml of sugar syrup on bee family. The duration of the experiment was 36 days.

The trace elements were added to the sugar syrup in the form of citrates water solution obtained from LLC "Nanomaterials and nanotechnology" in Kyiv and produced by the method of nanobiotechnology (M.V. Kosinov, V.G. Kaplunenko) [6]. For researches the samples of honey were selected - 10 g at the end of the research period from each bee colony. In samples of honey, the content of Fe, Cu, Zn, Co, Cr, Ni, Pb, Cd was determined on the atomic absorption spectrophotometer SF-115 PC with a computer program and its physical-chemical parameters of honey according to commonly accepted methods [11]. The statistical analysis of the results was carried out with the definition of average values, their deviations ($\pm m$) and the degree of probability (p).

Research results. Converting nectar to honey is a complex process that takes place under the influence of a number of physiological and physicochemical factors that cause quantitative and qualitative changes. The intensity of the processing nectar into honey largely depends on the number of breeding, natural and individual peculiarities of bee families, secretory activity of glands and a number of other factors [12]. The high nutritional value of honey is explained by the content of many biologically active substances, such as enzymes, organic acids, vitamins, aromatic lipid and nitrogenous compounds, trace elements.

According to the results of the study, it was marked that feeding various amounts of Ni and Co citrates with sugar syrup caused not similar differences in the content of separate trace elements in honey. In particular, Fe ($p < 0.001$), Cu and Zn content increase was noted in samples of honey of groups II and III bees. The intergroup differences in the content of Cd and Pb in samples of groups II and III compared to control (I) did not exceed the magnitudes of their mean statistical deviations.

1. Content of individual trace elements in polyphloric honey, mg / kg ($M \pm m$, $n=3$)

Trace elements	Bee colony groups		
	I stage (sugar syrup + Co citrate + Ni citrate)		
	I-control sugar syrup (SS)	II-experimental SS + Citrate Co	III-experimental Ca + Ni Citrate
Fe	4,5 \pm 0,02	5,7 \pm 0,06***	4,9 \pm 0,03***

Cu	0,44±0,04	0,51±0,06	0,55±0,02
Zn	0,54±0,07	0,66±0,02	0,60±0,04
Ni	0,25±0,05	0,24±0,03	0,21±0,02
Pb	0,08±0,02	0,05±0,02	0,07±0,02
Cd	0,04±0,006	0,03±0,006	0,05±0,005
Stage II (Sugar Syrup + Ag Citrate + Cu Citrate)			
	I-control sugar syrup (SS)	II-experimental SS + Ag Citrate + Cu Citrate	III-experimental SS + Ag Citrate + Cu Citrate
Fe	3,27±0,10	4,69±0,16***	2,24±0,04***
Cu	1,74±0,03	1,97±0,04**	2,11±0,26
Zn	1,71±0,12	1,13±0,10**	0,83±0,07**
Co	0,45±0,01	0,38±0,012**	0,47±0,02
Cr	0,43±0,006	0,47±0,01*	0,48±0,02
Pb	0,83±0,02	0,64±0,057*	0,56±0,02***
Cd	0,11±0,006	0,07±0,009**	0,06±0,008**

Note: in this and subsequent tables, the reliable difference between the experimental groups II and III compared to control group I; * - $p < 0,05$; ** - $p < 0,01$; *** - $p < 0,001$.

According to the results of the studies of complex feeding with sugar syrup, various doses of Ag and Cu citrates contributed to an increase in the Fe content ($p < 0,001$), and Cu in samples of honey II ($p < 0,01$) and not a significant decrease their in the III experimental groups.

Along with this, a probable decrease in Fe ($p < 0,001$) and Zn ($p < 0,01$) in the third experimental group compared to control was established. According to the results of our studies Pb and Cd content was lower in II ($p < 0,05-0,01$) and III ($p < 0,01-0,001$) experimental groups compared to the control.

As it's known a part of heavy metals contained in a nectar is "filtered" by bees after its gathering. This is connected with the fact that melliferous bees, that convert nectar into honey, repeatedly pass it through a chitin proboscis filter, which adsorbs a significant amount of heavy metals. That is why, in the conditions of repeated excess of the metals level in nectar and the organism of bees, honey is obtained with a small amount of them even from plants with high content of minerals. This can be explained by the special physiological ability of bees' organism to transform into only a certain fraction of any toxicant into products. Significant decrease of the heavy metals concentration in the process of nectar processing into honey is also largely determined by adsorption of them by the tissues of the bee organism with subsequent accumulation in organs that protect it from toxicosis [13].

Bee honey is one of the most complex natural products. More than four hundred different components were found in its content [14]. It's worth mentioning that chemical composition of honey is inconsistent and depends on the type of honey plants from which nectar was collected; the soil on which they grow; the time passed from the collection of nectar to the removal of honey from the honeycombs; time of honey storage; weather and climatic conditions. But the main groups of substances of the honey are constant [15].

The content of amino acids in natural honey is rather insufficient, but their correlation can play an important role in determining the biological value of honey. The researchers established that the greatest number of amino acid proline is contained in natural honey of melliferous bee. At the same time, it is known that the nectar of the flower of plants from which honey is formed does not have high content of proline. Consequently, bees excrete proline of their own metabolism into the honey, what indicate its biological value and quality.

Additional feeding of bees with sugar syrup and Co and Ni citrates caused fluctuations in the

proline amino acid content, which affects its biological value. In particular, the content of this amino acid in honey of group II bees was higher by 7.3% compared to the control group (Table 2). Similar differences were observed in Ag and Cu feeding conditions. Proline content in the II and III experimental groups was higher by 5.7% ($p < 0.05$) and 7.6% ($p < 0.01$), respectively, compared to control. Increase of proline content in honey is accompanied by a decrease in its pH, which increases its storage stability as well as fermentation resistance [16, 17].

2. Qualitative and physicochemical indices of polifloric honey ($M \pm m, n = 3$)

Bee colony groups	Quality Scores			
	Proline mg / kg	Diastase number, Gote number	Mass fraction of water, %	pH
I stage (sugar syrup + Co citrate + Ni citrate)				
I-control sugar syrup (SS)	301,89±9,09	15,58±0,14	19,27±0,06	4,26±0,005
II-experimental SS + Co Citrate	324,3±7,67	15,77±0,22	19,73±0,06**	4,38±0,003***
III-experimental SS + Ni Citrate	302,37±8,83	15,20±0,14	19,07±0,06	4,30±0,008***
II stage (Sugar Syrup + Ag Citrate + Cu Citrate)				
I-control sugar syrup (SS)	267,94±4,12	19,93±0,43	19,14±0,07	4,15±0,02
II experimental SS+ Ag Citrate + Cu Citrate	283,25±3,31*	20,36±0,44	19,00±0,10	4,24±0,05
III experimental SS + Ag Citrate + Cu Citrate	288,56±1,33**	20,98±0,47	19,74±0,07**	4,20±0,03***

According to the results of researches, somewhat higher indicators of honey diastase activity in stage II at feeding Ag and Cu citrates were established. This specific enzyme characterizes the activity of amyloid enzymes (amylase, diastase,), which indicates naturality, maturity and biological value of honey. Diastase number is the main indicator of the maturity and naturality of honey. It also depends on the breed of bees, the strength of the family and the species of honey carriers, from which the nectar was collected. In the honey samples of experimental group II (citrate Ag + citrate Cu) somewhat higher values of diastase number of honey were marked. It was higher by 2.39% (II group) and in III group by 5.85% compared to the control group, but the differences were unprobable. Thus, the addition of trace elements citrates with sugar syrup in the feeding of bees can influence the secretory activity of the digestive glands of bees during the processing of nectar into honey.

The mass fraction of water in honey met the current standards in both control and experimental groups. At the same time, in samples of honey of experimental groups a reliable decrease of hydrogen ions was indicated. In the 2nd experimental group (SS + citrate Co) ($p < 0,001$) and in the 3rd experimental group (SS + Ni citrate) ($p < 0,001$), (SS + citrate Ag+ citrate Cu) ($p < 0,001$).

Thus, changes in the qualitative composition of honey of experimental groups were accompanied by corrective action, concerning qualitative indices of experimental groups honey.

Conclusions

Feeding with sugar syrup of various amounts of Co, Ni, Ag, Cu citrates is noted for their synergistic and antagonistic effects on the level of individual mineral elements in samples of honey of melliferous bees. The probable differences in the lower content of Zn, Ni, Pb and Cd in the background of higher content of Fe, Cu and Cr in honey of experimental groups ($p < 0,05-0,001$) were established. The obtained results indicate positive changes in physicochemical parameters and the quality of honey with an increase in the content of proline and diastase number in the samples of both experimental groups. That confirms the expediency of using citrate supplementation of some trace elements Co, Ni, Cu та Ag in order to correct the mineral nutritional processes of melliferous bees and the quality of their products.

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