

IMPACT OF DIFFERENT FEED ON INTESTINE HEALTH OF HONEY BEES

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Abstract

Aim of study was to examine influence of different feed on epithelial cells of honey bees mid-gut. Study was conducted during autumn of 2012, in controled conditions, by using 48 mini laboratory cages with capacity of 150-200 bees each. For feeding of bees meadow, acacia and forest honey, molasses, acid and enzymatic invert syrup, with the addition of brewer's yeast and wort in certain proportions were used. Mid-gut with its content has been extracted from dead bees, histological preparation were made and then microscopically analyzed. Intestines were fixed in a 4% formaldehyde solution, inserted into paraffin blocks and cut with a microtome to 6 to 7 μm thick sections. Dewaxed sections were stained for general morphological purposes according to the Hemalaon-Eozinic method. The obtained results were compared with each other, and with the initial hypotheses about the impact of food on honeybee digestive tract structure. Also, within this framework of research, results of the effect of different foods on the life span of bees in this experiment will be presented.

INTRODUCTION

Honeybee meets their needs from the four natural sources: nectar, honey, pollen and water. These food sources are found in the environment, depending on the time of year and the current climatic conditions. The ability to rapidly and easily discover new food sources significantly affects the productivity of bee colonies, which is one of the important parameters of selection in beekeeping. Sometimes there is need of supplemental feeding of bees, which is prepared in two ways: using acid and enzymatic hydrolysis of sugar. Both of these chemical processes are in the service of a smaller physiological exhaustion of honeybees, given that it uses only simple sugars for its own needs. Residual acid in bee food causes splitting of its intestinal tract, with significantly shortening of bee's life. Enzymatic hydrolysis of sugar, with the help of enzymes sucrase-invertase, is a slower process, but without the harmful effects on the life of bees.

Brewer's yeast and wort, except that in its composition have sucrase-type enzymes invertase, are a rich source of protein, vitamins and minerals for bees. These components are very important meal for bee brood feeding, especially in the early springtime period, at a time of intense development of bee colonies.

LITERATURE REVIEW

The presence of fresh nectar and pollen has a positive effect on the development of the bee brood, and thus the strength of honey bee colonies and their subsequent productivity (Mladenovic et al., 1999). The quality of honey, as the main component of bee meals, depending on the origin of nectar, so, according to Komarov (1993), the largest ammounts of glucose and fructose (75%) and nitrogen compounds (0.97%) were found in a meadow honey, while honeydew was the richest in minerals (0.96%).

Kaftanoğlu et al. (2010) found that way of young larvae feeding, in terms of sugar content, determines further adult bees feeding and their sensitivity to the simple sugars level in food. According to Crailsheim (2010), protein level in nutrition is one of the main limiting factor for the bee colonies development, in terms of ability to fly, life expectancy and care for bee brood. Pollen is the main source of protein and contains essential acids necessary for bee's development, with ability to reduce their susceptibility to parasites and pathogens (Alaux et al., 2010). Crailsheim (2012) found that feed sources different to its composition and form (liquid or solid) are taken from bees of different ages, and that the same bees do not take more

than one form of food. Due to the absence of adequate natural food to provide winter food supplies, beekeepers use supplemental feeding of bees, mainly by sugar syrup (Dustmann et al., 1997; Ivanov, 1995). The sugar syrup can be enriched with supplements containing various concentrations of protein, vitamins, minerals, hormones and other substances necessary for growth and development of bees (Currie, 1999, Schmidt, 1999).

The most significant bee mortality was found during autumn supplemental feeding with acid hydrolysed invert syrup. Bee mortality can be caused by stomach poisoning or splitting of the digestive tract (Jachimowicz et al., 1975). Severson et al., (1984) conclude that the best carbohydrate source for supplementary feeding of bees was high fructose content syrup HFCS 55, which was confirmed by Rogers et al., (1992). According to their results, these syrups should be diluted to about 65% of solid content. Mladenović et al. (2002) suggest that different types of food for bees differentially affects the development and flourishing of bee society.

Dry brewer's yeast in nutrition significantly affects the longevity of bees. According to Taranov (2001), bees fed with dry brewer's yeast lived 38, and ones fed with a sugar syrup 22.5 days. The length of life of bees is affected by the amount of pollen in the hive (Rjamova, 1979). Thus, the amount of pollen in the hive to 4.4 kg can increase the life span of bees twice. For the development of a larva 145 mg of pollen is consumed, ie it is 1.5 kg of pollen for 10,000 bees (Peng et al., 1979). High quality replacement for pollen can completely replace the natural bee pollen in the diet, especially if compared to the impoverished sugar syrup, without protein supplements (Brodschneider et al., 2012). For example, brewer's yeast can be significant for the bee society development and increases the yield of honey, if a certain amount of egg yolk powder and soy flour is added and good mix provided (Abdellatif et al., 1971). According to Sahinler et al. (2003), combination of substitute for pollen and sugar syrup showed the best results. Crailsheim et al. (2006) found that proteins in artificial food for bees cross not just into the food reserves in the hive for immediately usage, but are also in so-called technical fit for feeding larvae and complete bee brood, until the moment of use.

MATERIAL AND METHODOLOGY

The subject and the work program

Study was carried out on the experimental apiary, in controlled conditions. The experiments were performed on indigenous race of bees "Kranjska" (*Apis mellifera carnica*) from the north-western part of Bosnia and Herzegovina. Experimental cages were filled with 200 bees, which were fed through two netted holes with prepared foods, according to experimental design. Dead bees were recorded on netted floor coverings. Daily measurements were done to determine the quantity of food consumed, as well as the number of dead bees.

In the experiment, we used the following sources of bee food:

- Acacia honey (control group),
- Sugar syrup,
- Acidic invert syrup (used tartaric acid)
- Enzymatic invert syrup.

Experimental groups of bees were fed in following variants:

- Diet only with syrup (sugar, acid and enzymatic)
 - Diet with syrup plus 40 g brewer's yeast,
 - Diet syrups with 40 gr beer wort,
 - Diet syrups with 20 gr brewer's yeast and 20 g beer wort,
- Each of the variant was repeated three times (three years of research).

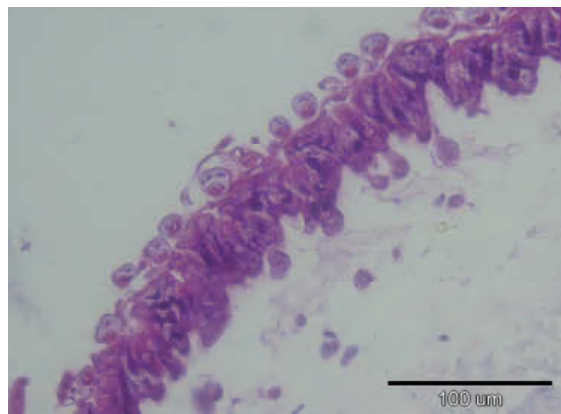
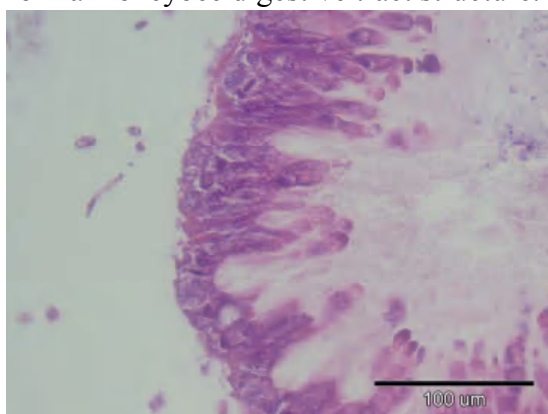
The objectives of the work were to determine:

- the optimal feed source for bees as a honey substitution,
- the effect of different feed to midgut epithelial layer of bees,
- the effect of different types of feed to the length of their lives.

RESULTS

Effect of different feed on digestive tract of bees

Mid-gut with its content has been extracted from dead bees, histological preparation were made and then microscopically analyzed. Intestines were fixed in a 4% formaldehyde solution, inserted into paraffin blocks and cut with a microtome to 6 to 7 μm thick sections. Dewaxed sections were stained for general morphological purposes according to the Hemalaon-Eozinic method. The obtained results were compared with each other, and with the normal honeybee digestive tract structure.



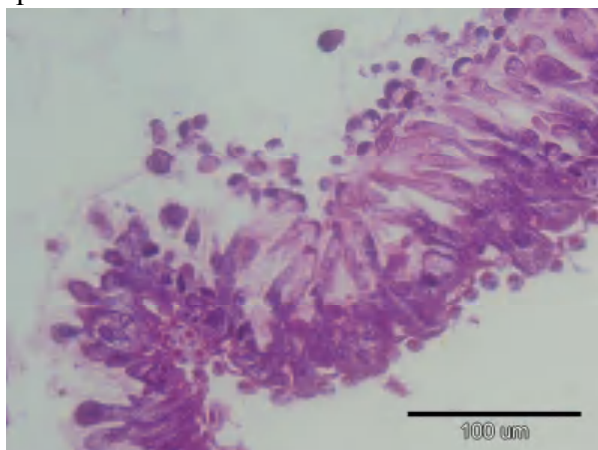
Pict.1. Middle gut of bee fed with accacia h. Pict. 2. Middle gut of bee fed with sugar syrup

When feeding bees with honey (acacia honey) we found:

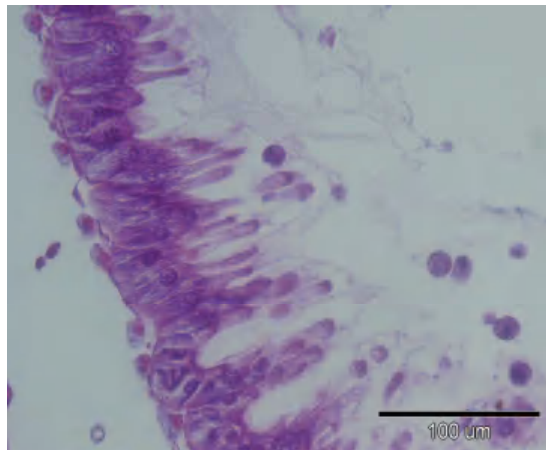
- Epithelial cells of the midgut are quite conservative, high are closely adherent to each other;
- Intestinal contents is quite homogeneous and compact, and fit close to the intestinal epithelium, which suggested well digested food.

When feeding sugar syrup, we found:

- Midgut wall is properly wrinkled, and in close contact with intestinal content;
- Epithelial cells are properly arranged on the inside intestinal wall;
- Slightly damaged top layer of epithelial cells was found, which is not present in all cells;
- Intestinal contents is not homogeneous, fairly loose consistency and lies directly on the intestinal epithelium.



Pict.3. Middle gut of bee fed with s.s. and yeast



Pict.4. Middle gut of bee fed with s. s. and beer wort

The influence of sugar syrup with yeast is reflected in the following:

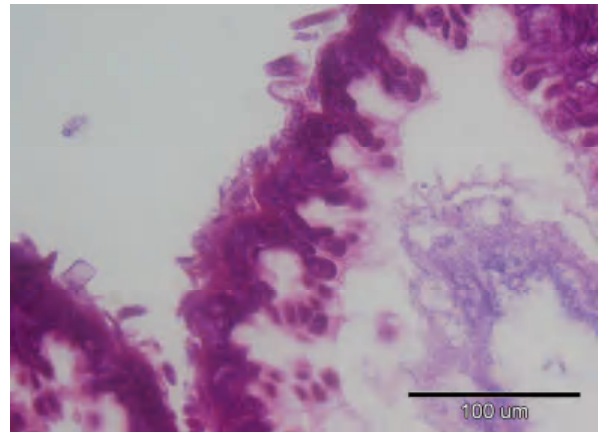
- Occurrence of spacing between individual epithelial cells and their not homogeneous distribution,
- As with sugar syrup diet, intestinal content is not homogeneous, fairly loose consistency and directly next to intestinal epithelium.

Feeding sugar syrup with beer wort has led to the following:

- Quite stretched bowel wall, leading to its reduced absorptive ability;
- Epithelial cells have damaged surface, while one part of the high epithelial cells missing, with only regenerative cells visible;
- Intestinal content is not homogeneous, fairly loose consistency and lies directly on the intestinal epithelium.



Pict.5.Middle gut of bee fed with s. s. yeast and beer wort



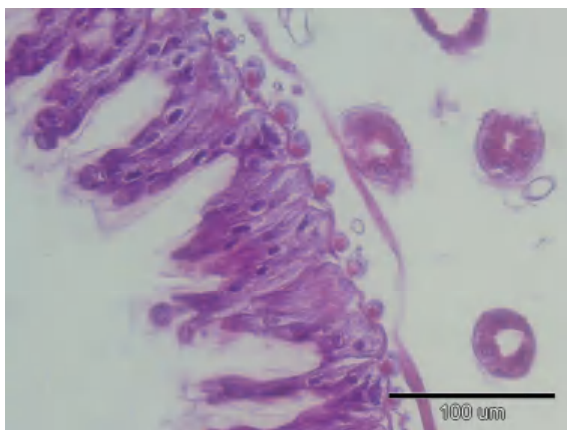
Pict.6.Middle gut of bee fed with acid inv. s.

When feeding bees with sugar syrup and a combination of yeast and wort we found:

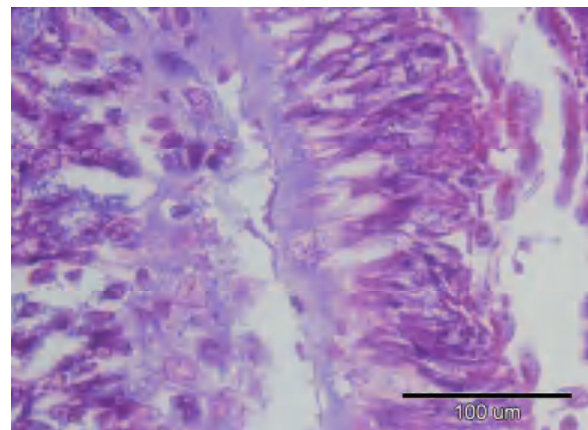
- Bowel wall quite stretched and thinned, so transverse wrinkles are not visible;
- Pronounced peak damage of epithelial cells, with their lack in intestinal epithelium (due to damage). Intestinal contents partially lies on the epithelium, with a good portion of the content compressed in the center of the intestine.

In midgut of bees fed with acidic invert syrup (tartaric acid) we found the following:

- Epithelial cells are widely separated, there are a number of gaps between them, which are manifested in the form of deep indentations in the epithelium;
- Almost totally damaged high columnar epithelial cells, with only regenerative cells not changed. Content in the intestinal lumen is rather loose consistency, with visible parts of disbanded cells.



Pict.7.Middle gut of bee fed with a. inv. s. and yeast



Pict. 8.Middle gut of bee fed with a. inv. s. and beer wort

In bees fed with acid invert syrup and yeast we found:

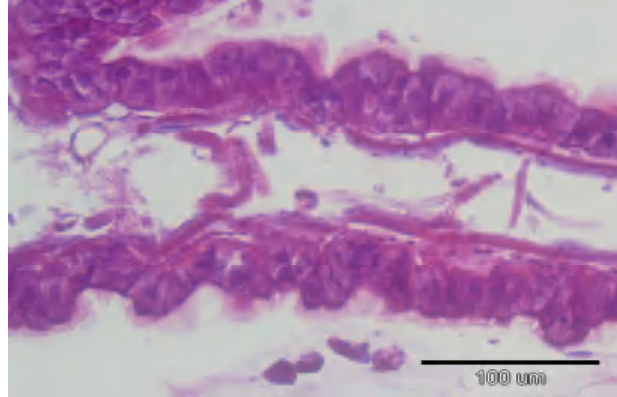
- A similar situation as with acid syrup, but with epithelial cells better preserved;
- there are sites of observed damage, which cover almost the entire depth of the epithelium.

In bees fed with acid invert syrup and beer wort we found:

- A complete layer of epithelial cells rather damaged by depth, but fairly compact and in close contact with intestinal content;
- Intestinal content of rather homogeneous consistence.



Pict.9. Middle gut of bee fed with a. inv. s., yeast and beer wort



Pict.10. Middle gut of bee fed with enz. inv. s.

In bees fed with acidic invert syrup and a combination of yeast and wort we found:

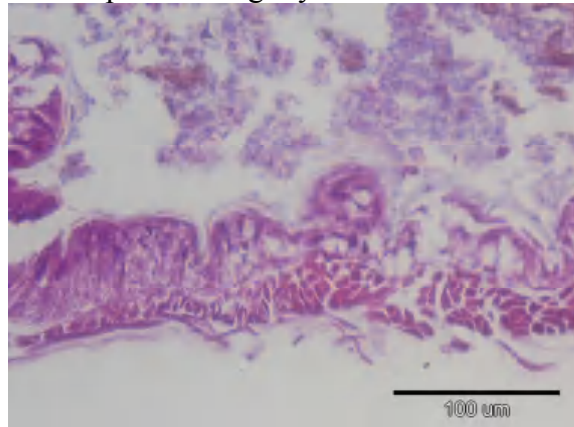
- About 2/3 of the total thickness of the epithelial layer is damaged on the entire surface of the lumen;
- Damaged epithelium is quite compact and homogeneous composition.

In bees fed with enzyme invert syrup we found:

- High columnar epithelial cells properly aligned on the basal membrane of the transverse corrugated pipes midgut;
- Partially separated peritrophic membrane which wraps some slightly loose bowel contents.



Pict.11. Middle gut of bee fed with enz. inv. syrup and yeast



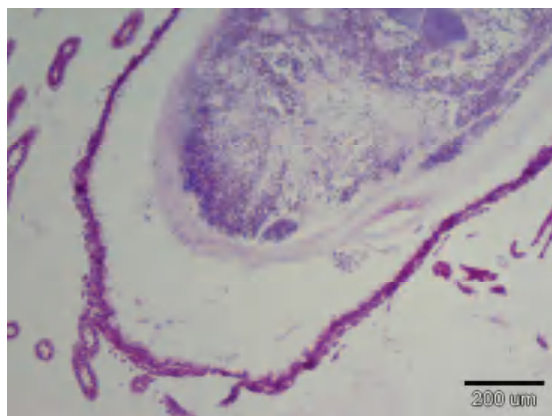
Pict.12. Middle gut of bee fed with enz. inv. s. and beer wort

In bees fed with enzyme invert syrup with the addition of yeast, we found the following:

- Slightly damaged surface epithelial cells and thereby expressed regenerative cells;
- Content in the lumen of the intestine is friable structure and directly adjacent to the midgut epithelium.

In bees fed with enzyme invert syrup and beer wort we found:

- in some places, the epithelial layer is totally damaged to the muscle layer;
- residual content in the intestinal lumen, organized in homogeneous clusters.



Picture 13. Middle gut of bee fed with enzyme invert syrup, yeast and beer wort

- In bees fed with enzyme invert syrup, yeast and beer wort we found following:
- Epithelial cells are deeply damaged throughout all gut surface;
 - Intestinal contents is compressed into a homogenous mass within the gut lumen, with a belted one homogeneous layer, it is difficult to digest for the bees.

The effect of feed source on the longevity of winter bees

As part of our research, we observed the influence of different feed types on the life span of bees. No other factors (number of bees in a cage, climatic conditions, etc.) had significant influence on this parameter of bee's body.

Table 1. Average life span of bees (days) in three years of study

Feed type	Life span of bees (days)			
	Year			
	1 st	2 nd	3 rd	<i>x</i>
H	26,55	29,46	25,15	27,05
SS	20,52	26,32	18,90	21,91
SS + Y	21,64	20,77	19,12	20,51
SS + BW	16,85	18,53	15,18	16,85
SS + Y + BW	17,46	18,82	18,82	18,37
AIS	10,30	15,17	10,98	12,15
AIS + Y	15,50	17,70	17,06	16,75
AIS + BW	16,59	18,76	16,82	17,39
AIS + Y + BW	16,44	18,92	18,26	17,87
EIS	24,98	26,82	19,43	23,74
EIS + Y	20,32	16,74	19,87	18,98
EIS + BW	15,38	20,00	15,91	17,10
EIS + Y + BW	20,06	18,69	18,89	19,21

* **H** - Honey; **SS** - Sugar Syrup; **Y** - Yeast; **BW** - Beers Wort; **AIS** - Acid Invert Syrup; **EIS** - Enzyme Invert Syrup

The shortest average life was found in winter bees fed with acid invert syrup (12.15 days), while the longest life was found in winter bees fed with honey (27.05 days). Natural food for bees - honey had a positive impact on the life span of bees, while acidic inverted feed significantly shortened the life of bees. This is also confirmed by the image the digestive tract of bees, as shown earlier part. Usage of tartaric acid for the process of complex sugars

inversion led to the splitting of the small intestine of bees and earlier bee mortality. Addition of brewers yeast and wort to primary sources of artificial food had different effects on the longevity of bees. Their adding to sugar syrup led to the shortening of life of bees in an average of 1.40 to 5.06 days. Similar results were found with their addition to enzyme syrups, where additions of yeast and wort led to shortening the average life of bees from 4.53 to 6.64 days. On the other hand, feed of additives to acidic invert syrup led to the prolonged average survival of bees from 4.60 to 5.72 days.

CONCLUSION

Based on our research, it can be concluded that feeding with different food sources has different influence on the digestive tract of bees, especially in the midgut epithelial layer. Natural source of food for bees - honey had no harmful effects on the midgut epithelial layer, and the intestinal contents were completely attached to this layer, which leads to the quality of digestion and maximum nutrient resorption. Similar results were got when feeding bees with sugar syrup and enzyme inverted syrup without the addition of yeast and malt. This means that each additon of yeast and malt lead to damage to the midgut epithelial layer, and the differences arise, depending on food source. The most serious damage on the epithelial layer was found in midgut of bees fed with acidic invert syrup (in all examined combinations).

Regarding the impact of different feed on the length of life of bees, it can be concluded that feeding with honey, enzyme invert sugar syrup had a positive effect on the life span of bees, while addition of brewer's yeast and wort shortens the life of bees, so recommendation is to use supplemental feeding without them, and that the use of these supplements should be more practiced during other seasons, especially if there is no natural pollen.

LITERATURE

1. Abdellatif M. A. F. H., El-Gair and N. M. Mohanna: "Three forms of yeast as a pollen substitute", Am. Bee J. 111, 14-15, 1971.
2. Alaux C., Dantec Christelle, Parrinello H., Le Conte Y.: "Nutrigenomics in honey bees", 4th Eurpoen Confernce of Apidology, Ankara, 81, 2010.
3. Brodschneider R., Haidmayer C., Crailsheim K.: "feeding pollen and pollen substitute to caged honey bees", COLOSS Workshop "Honey bee nutrition", Bled, 7, 2012.
4. Crailsheim K., Riessberger U., Thenius R., Kovac H., Vollmann J., Brodschneider R.: "Protein supplementation in honeybee colonies – where does the protein go?", The Second European Conference of ApidologyEurBee, 14, Prague, 2006.
5. Crailsheim K.: "The impact of nutritional protein on the honey bee – a review", 4th Eurpoen Confernce of Apidology, Ankara, 81, 2010.
6. Crailsheim K.: "Trophallaxis and protein nutrition of honey bees", COLOSS Workshop "Honey bee nutrition", Bled, 9, 2012.
7. Currie R. W.: "Problems associated with corn syrup", The XXXVIth Int. Apicultural Congress of Apimondia, Vancouver, 119-120, 1999.
8. Dustmann J. H., Wehling M., W. Von der Ohe : "Conversion of specific sugar solutions after their intake by honeybees", The XXXVth Int. Apicultural Congress of Apimondia, Antwerpen, 354, 1997.
9. Ivanov T. S., Ivanova T.: "A comparative study on feeding the honeybees with various kinds of carbohydrates", The XXXIVth Int. Apicultural Congress of Apimondia, Laussane, 306-308, 1995.

10. Jachimowicz T., Ei S. G., "Zur Problematik der Verwendung von Invertzucker für die Bienenfütterung", *Apidologie* 6 (2), 121-143; 1975.
11. Kaftanoglu O., Mustard A. Julie, Akyol E., Linksvayer A. T., Page Jr. R. E.: "Effect of carbohydrates on the development and sugar responsiveness of honey bees reared in vitro", 4th Eurpoen Confernce of Apidology, Ankara, 68, 2010.
12. Komarov A., A.: "Pčelovodstvo", Tula, Moskov, 1993.
13. Mladenović M., Mlađan V., Dugalić-Vrndić Nada: "Effect of a vitamin-mineral preparation on development and productivity of bee colonies", *Acta veterinaria*, 49, No 2-3, 177-184, 1999.
14. Mladenović M., Gajić D., Jevtić G., Mirjanić G.: "Efekat dodavanja obogaćenog sirupa na razvoj pčelinjih društava", *Biotehnologija u stočarstvu, XV Inovacije u stočarstvu*, Beograd, 2002.
15. Peng Y. S., Jay S. C.: "Larval rearing by worker honey bees lacking their mandibular glands. II Rearing by larger numbers of worker bees", *Can. Ent.* 111, 101-104, 1979.
16. Rjamova A. M.: "Značaj proljetne hrane", *Pčelovodstvo*, 5, Moskva, 1979.
17. Rogers, R. E. L., Illsley, E., "Alternative carbohydrate sources for feeding honey bees", *Plant Industry Branch Project Results, NSDA&M Annual Project Report*: 123-126; 1992.
18. Sahinler N., Sahin A., Kaya S.: "The effect of supplementary feeding on honeybee colony performance", *The XXXVIIIth Int. Apicultural Congress of Apimondia*, Ljubljana, 158, 2003.
19. Schmidt J. O.: "The nutritional mix and match of pollen: it isn't all the same for bees", *The XXXVIth Int. Apicultural Congress of Apimondia*, Vancouver, 123-124, 1999.
20. Severson, D. W., Erickson, Jr. E. L., "Honey bee (Hymenoptera: Apidae) colony performance in relation to supplemental carbohydrates", *J. Econ. Entomol.* 77(6): 1473-1478, 1984.
21. Taranov G. F.: "Hrana i prehranjivanje pčela", *Rossljhozizdat*, Moskva, 1986.