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DETERMINATION OF GLYCOGEN IN BEE ORGAN TISSUES

AS AN ENERGY METABOLISM PARAMETER

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SUMMARY

New method of glycogen determination in bee organ tissues, considerably different from the existing ones, was suggested. Ghoreishi's colorimetric method used today is time- labor- and cost-consuming as the major reagent – orcin – is quite expensive. Comparison of cost-effectiveness of the two methods demonstrated that the proposed technique makes it possible to reduce expenses by using a more available and less expensive resorcin instead of orcin and reducing total test time from 4 to 3 hours 5 minutes. Glycogen contents determination using the updated method was performed in bees of four breeds: Italian-Carpathian, Carpathian, Oka, gray Caucasus mountain honeybees. It was determined that the Oka honeybees demonstrated the highest glycogen level. It is known that the higher is glycogen contents in organs and tissues the better is energy metabolism in insects. Consequently, honeybees of Oka breeds have higher resistance to unfavorable weather conditions and can produce progeny with a higher level of immunity. So, the specified method of glycogen determination in bee organ tissues increases the accuracy of diagnosis and plays a very important role in determining the level of energy metabolism in insects and will be useful for apiculture.

Key words: bee, glycogen, bee organ tissues, biochemical tests, energy metabolism.

INTRODUCTION

Glycogen – a polysaccharide, or animal starch, which is synthesized in the body and deposited in all its organs and tissues [3, 4]. Glycogen is an easily mobilized reserve form of glucose and is a multichain polymer from blood glucose residues [2, 7].

There are several ways of glycogen determination in blood: periodic acid Schiff reaction (polysaccharides are detected in the result of a reaction that oxidizes alcohol groups that are converted to aldehyde groups and by identification of the latter by a color reaction with Schiff's reagent), the Shabadash method (in which the potassium periodate oxidizes glycogen to form aldehyde compounds that react easily with the Schiff reagent, cherry-violet staining is detected at the sites of glycogen localization), Ghoreishi's colorimetric method with orcin (glycogen is precipitated by alcohol, hydrolyzed to glucose in acidic medium and heated in sulfuric acid, which turns into hydroxymethylfurfural, condensing with orcin and forming a colored compound) [2, 3, 7].

The disadvantages of these methods are: time- and labor-consuming process, inaccurate glycogen determination in blood, and inability to determine its level in bee organ tissues. To improve the accuracy of diagnosis, reduce time and costs, it is necessary to improve the method of glycogen determination used in apiculture.

So, the development of method of glycogen determination in bee organ tissues is one of the urgent problems in beekeeping, since glycogen is an important indicator of the energy resource of insects [5, 6]. The purpose of the study was glycogen determination in organ tissues of bees of different breeds.

MATERIALS AND METHODS

Four groups of bees of different breeds were used for the study (Italian-Carpathian, Carpathian, Oka, gray Caucasus mountain honeybees), 10 bees in each group.

The study contained the following steps.

Preparation of extract from bee organ tissues. With the help of forceps and eye scissors, the integument and intestines of bees were removed. Tissues and organs obtained from ten bees were ground in a porcelain mortar, adding physiological solution (1: 1). The suspension was then kept in the refrigerator at + 5 ° C for 1.0-1.5 h and filtered through a paper filter.

Table 1
Extinction parameters

Groups of bees	Extinction of samples	
	E_{ts}	E_{ss}
group 1 (Italian-Carpathian breed)	0.438	0.256
group 2 (Carpathian breed)	0.584	0.251
group 3 (Oka breed)	0.950	0.301
group 4 (gray Caucasus mountain breed)	0.566	0.253

Fig. 1. Extinction parameters

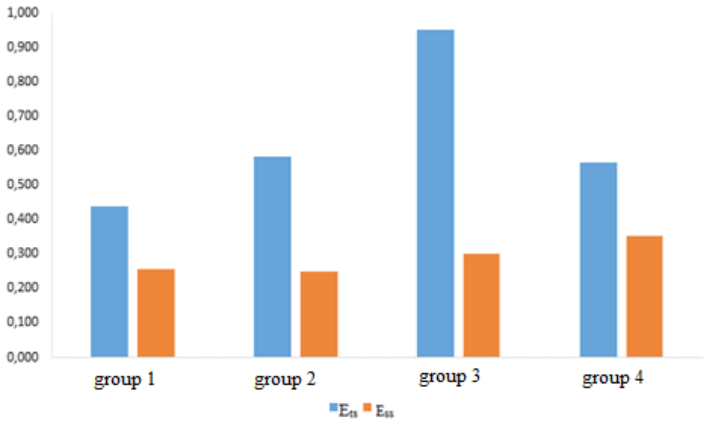
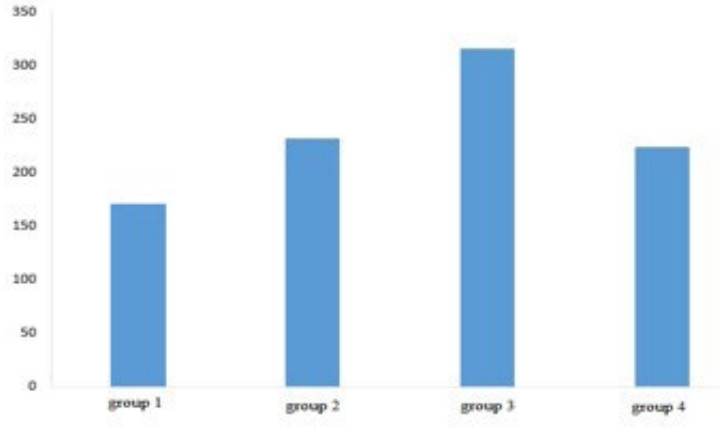


Table 2
Glycogen content in extracts from bee organ tissues

Groups of bees	Glycogen content, mg%
group 1 (Italian-Carpathian breed)	171.0
group 2 (Carpathian breed)	232.0
group 3 (Oka breed)	316.0
group 4 (gray Caucasus mountain breed)	224.0

Fig. 2. Glycogen content in samples



Preparation of solutions. A 1% resorcin solution was prepared with 52% sulfuric acid; 5% solution of trichloroacetic acid was prepared by dissolving a sample of 5 g in 99 ml of distilled water. To obtain a standard solution, 10 mg of glucose was dissolved in 300 ml of distilled water.

Precipitation and hydrolysis of glycogen from an extract from bee organ tissues. 0.3 ml of the filtered extract from the bee tissues were placed in a centrifuge tube, 0.2 ml of distilled water and 0.2 ml of 30% sodium hydroxide solution were added. The tube was then placed in a boiling water bath for 1.5 hours, cooled, 1 ml of 96% ethyl alcohol was added, mixed, cooled on ice for 15 min and centrifuged at 3000 g for 15 min. The supernatant was removed, and 1 ml of 96% ethyl alcohol was added to the precipitate, resuspended, cooled on ice for 15 minutes and centrifuged again at 3000 g for 15 min. The supernatant was carefully removed, the pellet was resuspended in 3 ml of distilled water, 13 ml of 52% sulfuric acid solution and 2 ml of 1% resorcin were added and mixed.

Determination of glycogen content. 3 ml of distilled water and 13 ml of 52% sulfuric acid solution were added to the control (clean) tube and to the tube with a standard solution of glucose, mixed, and 2 ml of 1% resorcin solution were added to them.

All tubes were placed in a water bath for 20 minutes at a temperature of 80 ° C, then cooled on ice for 15 minutes. The tubes containing glucose had a brown-yellow color.

Photocolorimetry was performed using CPK-3 at a wavelength of l = 315 nm.

Calculation of the glycogen content in the obtained extract was carried out according to the formula:

$$G = \frac{E_{ts}}{E_{ss}} \times 100,$$

where G – amount of glycogen, mg%;
E_{ts} – extinction of the test sample;
E_{ss} – the extinction of a standard solution.

The calculation of the economic efficiency of the proposed method was carried out according to generally accepted formulas for calculating the costs of carrying out activities, which are made up of the cost of labor and material resources used to conduct research [1].

RESULTS AND DISCUSSION

Summary data for the definition of extinction are given in Table 1 and in Figure 1.

In all four groups of bees, the determined parameter varied significantly. The results of the study demonstrated that the Oka honeybees demonstrated the highest glycogen level (both in the test sample and in the standard solution) and amounted to 0.950 and 0.301, respectively. The lowest level of extinction was shown by the bees of Italian-Carpathian breed: 0.438 in the test sample and 0.256 in the standard solution.

Glycogen content in the obtained extracts was calculated for all groups of bees. Summary data are given in Table 2 and in Figure 2.

The presented data demonstrate that different bee breeds have different level of glycogen content, and different energy resources. It depends on specific differences of bee organisms.

It was determined that the Oka honeybees (group 3) demonstrated the highest glycogen level. Consequently, bees of this breed have better energy metabolism. Honeybees of Oka breeds have higher resistance to un-

favorable weather conditions and can produce progeny with a higher level of immunity

The calculation of the economic efficiency of the presented method was also carried out in comparison with the most frequently used method for the determination of glycogen according Ghoreishi's method with orcin (Table 3).

The data given in Table 3 demonstrate that high economic efficiency of the proposed method is achieved by:

– reducing cooling time from 30 to 15 minutes after boiling in water bath by quick cooling in freezer;

– reduced number of centrifugations;

– using more available and less expensive resorcin instead of orcin;

– reducing total test time from 4 to 3 hours 5 minutes.

Table 3
Economic efficiency of the method for glycogen determination in bee organ tissues

Parameters	Ghoreishi's method	Method for glycogen determination in bee organ tissues
Number of centrifugations at 3000 g for 15 min	3	2
Major chemical reagent	Orcin 1%	Resorcin 1%
Reagent cost per 1 kg, in roubles.	3960	723
Total test time	4 hours	3 hours 5 minutes

CONCLUSION

The proposed method is available, simple-to-use and enables to determine glycogen content in bee organ tissues more quickly than by known methods for glycogen content determination in animal blood.

The proposed method enables to calculate glycogen content in extract from bee organ tissues and to determine the level of energy metabolism in bees of different breeds for identification of breeds which have higher resistance to unfavorable weather conditions and which can produce progeny with a higher level of immunity.

The proposed method is characterised by high economic efficiency if compared with the existing methods of glycogen determination.

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